ACTA OBSTETRICIA ET GYNECOLOGICA SCANDINAVICA

Vol. XLVII

LUND 1968 BERLINGSKA BOKTRYCKERIET

ACTA OBSTETRICIA ET GYNECOLOGICA SCANDINAVICA

Vol XLVII

LUND 1968. BERLINGSKA BOKTRYCKERIET

TABLE OF CONTENTS

VOL. XLVII

A

B

C

D

E

F

G

H

I

J

F

G

H

I

J

T

W

O

P

R

S

T

W

Anderson, Anne B. M. and Turnbull, Alexander C. Acta obst. et gynec. scandinav. 47 1, 1968

From the Clinical Research Unit Department of Obstetrics University of Aberdeen Aberdeen Maternity Hospital Foresterhill Aberdeen Scotland

CHANGES IN AMNIOTIC FLUID SERUM AND URINE FOLLOWING THE INTRA AMNIOTIC INJECTION OF HYPERTONIC SALINE

BY

ANNE B. M. ANDERSON AND ALEXANDER C. TURNBULL

The intra-amniotic injection of hypertonic saline is now a well known technique for inducing abortion (*Bengtsson* and *Csapo* 1962 *Wiqvist* and *Eriksson* 1964 *Turnbull* and *Anderson* 1965) However little is known of the distribution of the sodium chloride injected or its effect on the maternal body fluids.

Some of the hypertonic saline may be inadvertently injected into the maternal circulation rather than into the amniotic fluid and this can prove fatal (*Wagatsuma* 1965) Two maternal deaths following intra-amniotic injection of hypertonic saline described recently (*Cameron* and *Dayan* 1966) may have been the result of this complication for they were associated with widespread cerebral infarction.

The effects on the mother of saline injected into the amniotic sac are not well understood. An attempt to correlate the changes in electrolyte concentration in amniotic fluid, serum and urine was made by *King, Friedman* and *Steer* (1964) who reported that, although radioactive sodium injected into the amniotic sac could be detected in the maternal blood within 30 minutes, there was little or no change in maternal serum sodium concentration. In the single case they studied, however only 100 ml. of hyper tonic saline was injected (one-half the amount usually required to induce abortion) and the pregnancy was terminated by abdominal hysterotomy before uterine contractions had started. *Weingold Seigal* and *Stone* (1965) could not detect any change in the serum sodium and chloride in the 24-hour period after

Z

Å

Ö

Anderson, Anne B. M. and Turnbull, Alexander C. Acta obst. et gynec. scandinav. 47, 1, 1968

From the Clinical Research Unit, Department of Obstetrics, University of Aberdeen, Aberdeen Maternity Hospital, Foresterhill, Aberdeen, Scotland

CHANGES IN AMNIOTIC FLUID, SERUM AND URINE FOLLOWING THE INTRA-AMNIOTIC INJECTION OF HYPERTONIC SALINE

BY

ANNE B. M. ANDERSON AND ALEXANDER C. TURNBULL

The intra-amniotic injection of hypertonic saline is now a well known technique for inducing abortion (*Bengtsson* and *Csapo* 1962, *Wiqvist* and *Eriksson* 1964, *Turnbull* and *Anderson* 1965). However little is known of the distribution of the sodium chloride injected or its effect on the maternal body fluids.

Some of the hypertonic saline may be inadvertently injected into the maternal circulation rather than into the amniotic fluid and this can prove fatal (*Wagatsuma* 1965). Two maternal deaths following intra-amniotic injection of hypertonic saline described recently (*Cameron* and *Dayan* 1966) may have been the result of this complication for they were associated with widespread cerebral infarction.

The effects on the mother of saline injected into the amniotic sac are not well understood. An attempt to correlate the changes in electrolyte concentration in amniotic fluid, serum and urine was made by *King, Friedman* and *Steer* (1964) who reported that, although radioactive sodium injected into the amniotic sac could be detected in the maternal blood within 30 minutes, there was little or no change in maternal serum sodium concentration. In the single case they studied, however, only 100 ml. of hypertonic saline was injected (one-half the amount usually required to induce abortion) and the pregnancy was terminated by abdominal hysterotomy before uterine contractions had started. *Weingold, Seigal* and *Stone* (1965) could not detect any change in the serum sodium and chloride in the 24-hour period after

intra-amniotic saline injection but found that these electrolytes were rapidly excreted in the urine On the other hand *Pinkerton* (1966) found no evidence that hypertonic saline or penicillin injected into the amniotic fluid passed out into the maternal circulation.

This paper reports the findings of a systematic study of the sequential changes in amniotic fluid and in maternal body fluids following the injection of hypertonic saline. A brief preliminary report was published (*Turnbull* and *Anderson* 1966) when it became clear that the changes could be dangerous in patients with cardiac or renal disease

Material

The studies were done on 7 healthy young women of differing parity with normal cardiovascular and renal function. In all patients therapeutic abortion was undertaken for psychiatric reasons at 16–20 weeks gestation.

Induction of abortion was successful and uncomplicated in all but one patient who developed an intra-uterine infection which responded quickly to antibiotics All foetuses were dead and macerated when delivered.

Methods

The technique of transabdominal amniocentesis and insertion of a polyethylene catheter into the amniotic sac has been described elsewhere (*Turnbull* and *Anderson* 1965) The catheter was left in situ until abortion occurred to facilitate collection of samples of amniotic fluid

In each case 200 ml. of amniotic fluid was aspirated and 200 ml of 20 per cent sodium chloride solution injected.

An indwelling Foley catheter allowed complete emptying of the bladder before amniocentesis and the collection of urine at fixed intervals during the process of abortion.

The concentration of sodium and potassium was measured in serial samples of amniotic fluid serum and urine with the Unicam SP 900 *flame* spectrophotometer

Packed Cell Volume was estimated in venous blood samples

taken with minimum stasis. Capillary tubes checked for even taper were used, spun in a Hawksley Micro-Haematocrit Centrifuge at 13,000 revs./min. for 5 minutes and then read, in duplicate at the black line. No correction was made for trapped plasma.

Osmolality was measured by freezing-point depression using the Advanced Instruments Osmometer

Antidiuresis was measured by noting changes in urinary output during water loading. A steady diuresis was induced by intravenous infusion of 5 per cent dextrose at 1080 ml/hr in one patient and 800 ml/hr in a second. The bladder was completely emptied each 15 minutes by means of an indwelling self-retaining catheter Urine flow in ml/minute was calculated for each 15-minute urine collection and osmolality measured in each sample When a steady urine flow had been achieved, intravenous vasopressin was given for 30 minutes at a rate of 20 mU/min. in one patient, 40 mU/min. in the other using the Continuous Slow Injector (Palmer Ltd., London) The blood pressure level during the vasopressin infusion did not rise above 136/90 mm. Hg in the patients studied.

Results

Changes in *Amniotic Fluid Electrolytes*

Tables I and II and Figs. 1 and 4 show the concentration of electrolytes in serial samples of amniotic fluid taken in 2 patients (A and B) before and after the injection of hypertonic saline, up to the time of abortion. Sodium was estimated in both patients but potassium could not be measured in the first patient because the aspirated fluid was blood-stained. In both patients the membranes remained intact throughout the study

Tables I and II show that before the saline injection the concentration of sodium and potassium in amniotic fluid was lower than in maternal serum. The total amount of sodium chloride injected into each amniotic sac (200 ml. of 20 per cent solution) was 683 milli-equivalents of both sodium and chloride. The sodium concentration in the amniotic fluid 1 hour after the saline injection was 2200 and 2160 mEq/L in patients A and B

intra-amniotic saline injection, but found that these electrolytes were rapidly excreted in the urine On the other hand, *Pinkerton* (1966) found no evidence that hypertonic saline or penicillin injected into the amniotic fluid passed out into the maternal circulation

This paper reports the findings of a systematic study of the sequential changes in amniotic fluid and in maternal body fluids following the injection of hypertonic saline A brief preliminary report was published (*Turnbull* and *Anderson* 1966) when it became clear that the changes could be dangerous in patients with cardiac or renal disease

Material

The studies were done on 7 healthy young women of differing parity with normal cardiovascular and renal function. In all patients therapeutic abortion was undertaken for psychiatric reasons at 16–20 weeks gestation.

Induction of abortion was successful and uncomplicated in all but one patient who developed an intra-uterine infection which responded quickly to antibiotics. All foetuses were dead and macerated when delivered.

Methods

The technique of transabdominal amniocentesis and insertion of a polyethylene catheter into the amniotic sac has been described elsewhere (*Turnbull* and *Anderson* 1965) The catheter was left in situ until abortion occurred to facilitate collection of samples of amniotic fluid.

In each case 200 ml of amniotic fluid was aspirated and 200 ml of 20 per cent sodium chloride solution injected.

An indwelling Foley catheter allowed complete emptying of the bladder before amniocentesis and the collection of urine at fixed intervals during the process of abortion.

The concentration of sodium and potassium was measured in serial samples of amniotic fluid, serum and urine with the Unicam SP 900 flame spectrophotometer

Packed Cell Volume was estimated in venous blood samples

taken with minimum stasis. Capillary tubes, checked for even taper were used, spun in a Hawksley Micro-Haematocrit Centrifuge at 13 000 revs./min. for 5 minutes and then read, in duplicate at the black line. No correction was made for trapped plasma.

Osmolality was measured by freezing-point depression using the Advanced Instruments Osmometer

Antidiuresis was measured by noting changes in urinary output during water loading. A steady diuresis was induced by intravenous infusion of 5 per cent dextrose at 1080 ml/hr in one patient and 800 ml/hr in a second. The bladder was completely emptied each 15 minutes by means of an indwelling self-retaining catheter Urine flow in ml/minute was calculated for each 15-minute urine collection and osmolality measured in each sample. When a steady urine flow had been achieved, intravenous vasopressin was given for 30 minutes at a rate of 20 mU/min. in one patient 40 mU/min. in the other using the Continuous Slow Injector (Palmer Ltd. London) The blood pressure level during the vasopressin infusion did not rise above 136/90 mm. Hg in the patients studied.

Results

Changes in Amniotic Fluid Electrolytes

Tables I and II and Figs 1 and 4 show the concentration of electrolytes in serial samples of amniotic fluid taken in 2 patients (A and B) before and after the injection of hypertonic saline, up to the time of abortion. Sodium was estimated in both patients but potassium could not be measured in the first patient because the aspirated fluid was blood-stained. In both patients the membranes remained intact throughout the study

Tables I and II show that before the saline injection the concentration of sodium and potassium in amniotic fluid was lower than in maternal serum. The total amount of sodium chloride injected into each amniotic sac (200 ml. of 20 per cent solution) was 683 milli-equivalents of both sodium and chloride. The sodium concentration in the amniotic fluid 1 hour after the saline injection was 2200 and 2160 mEq/L in patients A and B

intra-amniotic saline injection but found that these electro were rapidly excreted in the urine On the other hand *PinLe* (1966) found no evidence that hypertonic saline or peni *injected into the amniotic fluid passed out into the mat* circulation.

This paper reports the findings of a systematic study of sequential changes in amniotic fluid and in maternal body fl following the injection of hypertonic saline. A brief prelimi report was published (*Turnbull* and *Anderson* 1966) whe became clear that the changes could be dangerous in pati with cardiac or renal disease.

Material

The studies were done on 7 healthy young women of diffe parity with normal cardiovascular and renal function. In patients therapeutic abortion was undertaken for psychia reasons at 16–20 weeks gestation.

Induction of abortion was successful and uncomplicated in but one patient who developed an intra-uterine infection wh responded quickly to antibiotics All foetuses were dead macerated when delivered

Methods

The technique of transabdominal amniocentesis and insertion a polyethylene catheter into the amniotic sac has been describ elsewhere (*Turnbull* and *Anderson* 1965) The catheter w left in situ until abortion occurred to facilitate collection samples of amniotic fluid.

In each case 200 ml of amniotic fluid was aspirated and 200 r of 20 per cent sodium chloride solution injected

An indwelling Foley catheter allowed complete emptying of t bladder before amniocentesis and the collection of urine fixed intervals during the process of abortion.

The concentration of sodium and potassium was measured serial samples of amniotic fluid, serum and urine with the Unica SP 900 flame spectrophotometer

Packed Cell Volume was estimated in venous blood sample

13	480	155	3.60	32.3	293	61	25.5	6.5	801
14						90	34.3	6.6	809
15						55	43.9	6.9	828
16	520					51	55.7	6.9	847
17						53	68.9	7.1	857
18	490	155	3.40	30.8	279	60	84.2	7.2	847
19						45	95.0	7.0	853
20	480					62	109.4	7.9	823
21						45	116.3	8.4	849
22						36	120.9	8.9	829
23	440	160	3.25	30.6	277	58	128.8	8.9	765
24						38	134.9	8.2	806
25						50	143.0	7.5	802
26	350	150	3.40	30.9	278	32	148.9	6.5	794
27						62	163.8	6.5	66
28						70	180.9	7.0	778
29	320	140	3.65	30.1	275	37	189.1	6.3	796
30						49	199.1	5.7	798
31						69	15.4	6.0	723
32						162	253.1	8.9	596
33	280	147		31.6		93	278.1	9.9	638
34						26	288.3	8.9	703
35	260	150	3.15	32.4	276	120	329.2	9.3	41
36						151	375.0	10.4	674
37						121	410.3	12.2	682
38	230					103	440.8	13.5	683
39[a]		140		32.8		110	468.2	15.8	606

[a] 30 minutes after abortion

Table I *Changes in Amniotic Fluid Serum and Urine Before and After Intra-Amniotic Infection of Hypertonic Saline*

Patient A

Time (hours)	Amniotic Fluid Na. mEq/l.	Serum				Urine			
		Na. mEq/l.	K. mEq/l	P C V %	Osmolality m.osmols./kg.	Volume ml.	Na. Excess mEqs. cumulative totals.	K. Excess mEqs. cumulative totals.	Osmolality m.osmols./kg.
Before Saline									
3						81			255
2		143	3 85	33 5	282	40			579
1	134	147	3 75	33.5	280	37			643
After Saline									
1	2200	147	4 15	33 4	281	37	0.0	0.3	673
2	1820	160	3 90	32.9	285	36	0.9	0.9	718
3	1430	160	4 10	33 1	284	24	0.9	1 2	730
4	1300	143	3 90	32.8	290	22	1.3	1 6	770
5	1320	143	3.85	32 3	296	22	1 4	2.1	771
6						25	1 4	2.3	753
7	1400	147	3.65	31.5	292	22	1 6	2.3	742
8						30	3.3	2.9	792
9						38	7.0	4.3	762
10	720	150	3 50	32.2	290	38	10.3	5 4	785
11						37	13.3	5.9	785
12						42	18.4	6 0	771

13	480	155	3.60	32.3	293	61	25.5	6.5	801
14						50	34.3	6.6	809
15						55	43.8	6.9	828
16	520					51	55.7	6.9	847
17						53	68.9	7.1	857
18	490	155	3.40	30.8	279	60	84.2	7.2	847
19						45	95.0	7.0	853
20	480					62	109.4	7.9	823
21						45	116.3	8.4	849
22						38	120.9	8.9	829
23	440	160	3.25	30.6	277	58	128.8	8.9	785
24						36	134.9	8.2	806
25						50	143.0	7.5	802
26	350	150	3.40	30.9	278	32	148.9	6.5	794
27						62	163.8	6.5	786
28						70	180.9	7.0	778
29	320	140	3.65	30.1	275	37	189.1	6.3	796
30						49	199.1	5.7	798
31						69	215.4	6.0	723
32						162	253.1	8.9	596
33	280	147		31.6		93	278.1	9.9	638
34						26	288.3	8.9	703
35	260	150	3.15	32.4	276	120	329.2	9.3	741
36						151	375.0	10.4	674
37						121	410.3	12.2	682
38	230					103	440.8	13.5	683
39		140		32.8		110	468.2	15.8	606

30 minutes after abortion

Table II. Changes in *Amniotic Fluid Serum and Urine Before and After Intra-Amniotic Injection of Hypertonic Saline*

Patient B

Time (Hours)	Amniotic Fluid		Serum				Urine			
	Na. mEq/L	K. mEq/l	Na. mEq/l	K. mEq/L	P.C.V %	Osmolality m.osmols/kg.	Volume ml.	Na. Excess mEq cumulative totals.	K. Excess mEq cumulative totals	Osmolality m.osmols./kg.
Before Saline										
3			134	3.85	32 4	279	63			567
2			132	3.90	32 9	278	40			625
1	132	3 75	134	3 90	32 5	280	40			692
After Sali e										
1	2160	3 55	140	3 65	32.7	285	26	0 0	0 0	750
2	1000	3.60	144	3.85	32.4	293	60	0 0	0 4	738
3	950	4.20	144	4 15	31.8	295	52	1.9	1 4	732
4							92	19 6	6.9	743
5	680	4.80	140	4 05	31 6	295	67	29.9	8 4	735
6							98	48.2	10.8	746
7	650	5 40					110	76 5	13.7	743
8							101	107 7	15.5	767
9	560	5 40	137	3 70	30.5	290	35	112.6	14.8	760
10							93	133 1	16 1	797
11	440	4.80					39	140 5	15.5	828

12			144	3.90	30.0	287	50	151.5	15.0	818
13	419	5.40					37	158.2	14	797
14							87	185.8	15.2	791
15	390	4.20					48	193.9	14.8	783
16							50	203.9	14.6	808
17	370	4.80	144	4.25	31.3	283	45	212.2	14.1	797
18							69	228.7	14.3	825
19	260	4.20					59	41.9	14.1	820
20							74	260.0	14.3	818
21	250	4.80	137	3.60	31.2	281	89	287.6	15.4	820
22							64	301.1	15.7	808
23							94	324.9	16.4	806
24							44	332.0	15.5	792
25	210	3.00	134	3.35	32.2	277	81	350.9	16.0	810
26							73	366.7	16.2	807
27	200	2.40					62	379.5	17.1	774
28							27	380.5	16.8	774
29			137	3.35	31.8	276	40	385.0	17.5	801
30							24	384.9	17.3	809
31	190	2.40	144	3.55	32.3	275	35	387.5	17.7	882
32							38	388.6	19.4	827
33							41	389.4	21.5	850
34	160	2.40	147	3.60	32.5	274	28	387.7	22.6	793
35							80	389.8	28.8	715

30 minutes after abortion

Table II. *Changes in Amniotic Fluid Serum and Urine Before and After Intra-Amniotic Injection of Hypertonic Saline*

Patient B

Time (Hours)	Amniotic Fluid		Serum				Urine			
	Na. mEq/L	K. mEq/l	Na. mEq/L	K. mEq/L	P.C.V %	Osmolality m.osmols./kg.	Volume ml.	Na. Excess mEq cumulative totals	K. Excess mEq cumulative totals.	Osmolality m osmols./kg.
Before Saline										
3			134	3.85	32.4	279	63			567
2			132	3.90	32.9	278	40			625
1	132	3.75	134	3.90	32.5	280	40			692
After Saline										
1	2160	3.55	140	3.65	32.7	285	26	0.0	0.0	750
2	1000	3.60	144	3.85	32.4	293	60	0.0	0.4	738
3	950	4.20	144	4.15	31.8	295	52	1.9	1.4	732
4							92	19.6	6.9	743
5	680	4.80	140	4.05	31.6	295	67	29.9	8.4	735
6							98	48.2	10.8	746
7	650	5.40					110	76.5	13.7	743
8							101	107.7	15.5	767
9	560	5.40	137	3.70	30.5	290	35	112.6	14.8	760
10							93	133.1	16.1	797
11	440	4.80								

12			144	3.90	30.0	287	50	151.5	15.0	819
13	410	5.40					37	158.2	14.2	797
14							87	185.8	15.2	791
15	390	4.20					46	193.9	14.8	783
16							50	203.9	14.6	806
17	370	4.80	144	4.25	31.3	283	45	212.2	14.1	797
18							69	228.7	14.3	825
19	260	4.20					59	241.8	14.1	820
20							74	260.0	14.3	818
21	250	4.80	137	3.60	31.2	281	89	287.6	15.4	820
22							64	301.1	15.7	808
23							94	324.9	16.4	806
24							44	332.0	15.5	792
25	210	3.00	134	3.35	32.2	277	81	350.9	18.0	810
26							73	368.7	18.2	807
27	200	2.40					62	379.5	17.1	774
28							27	380.5	16.9	774
29			137	3.35	31.8	276	40	385.0	17.5	801
30							24	384.9	17.3	809
31	190	2.40	144	3.55	32.3	275	35	387.5	17.7	882
32							38	388.6	19.4	827
33							41	389.4	21.5	850
34	160	2.40	147	3.60	32.5	274	28	387.7	22.6	793
35							80	389.8	28.8	715

30 minutes after abortion.

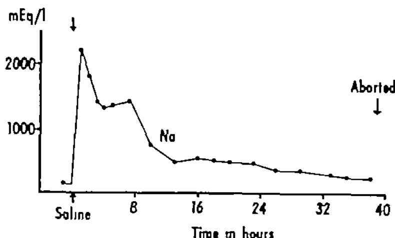


Fig 1 Patient A. Changes in amniotic fluid following intra amniotic injection of hypertonic saline

respectively Assuming complete mixing, the estimated volume of amniotic fluid would be approximately 300 ml Sodium con centration fell rapidly and 12 hours after injection was 400–500 mEq/l Thereafter the level fell more slowly but never returned to that found before the saline injection values of 230 and 160 mEq/l being found in the two patients just before the uterine contents were expelled

Potassium concentration in patient B rose from an initial value of 3 75 mEq/l to a level of 5 40 mEq/l 7 hours after the hyper tonic saline injection was maintained at this level until 13 hours after saline and then began to fall reaching the low value of 2 40 mEq/l just before abortion None of the amniotic fluid samples was contaminated with blood.

Changes in Serum

The results in patients A and B are shown in Tables I and II and in Figs 2 and 5 An attempt was made to standardise fluid intake in these patients at approximately 100 ml /hour

Serum sodium showed a biphasic pattern in patient A for it reached a first peak of 160 mEq/l. only 2 hours after the saline injection, was maintained at this level during the third hour

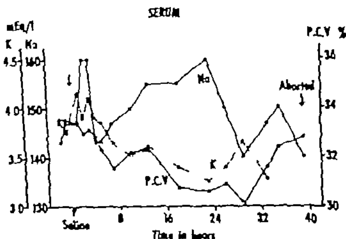


Fig. 2. Patient A. Changes in serum following intra-amniotic injection of hypertonic saline.

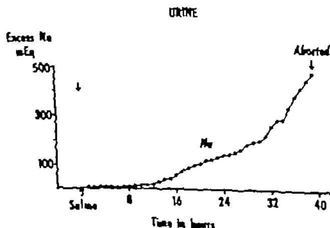


Fig. 3. Patient A. Changes in urine following intra-amniotic injection of hypertonic saline.

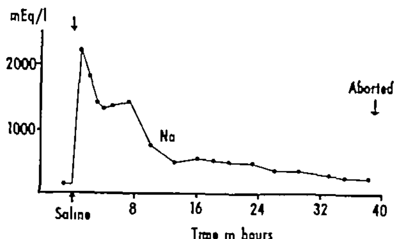


Fig 1 Patient A. Changes in amniotic fluid following intra-amniotic injection of hypertonic saline

respectively Assuming complete mixing the estimated volume of amniotic fluid would be approximately 300 ml. Sodium concentration fell rapidly and 12 hours after injection was 400–500 mEq/l Thereafter the level fell more slowly but never returned to that found before the saline injection values of 230 and 160 mEq/l being found in the two patients just before the uterine contents were expelled.

Potassium concentration in patient B rose from an initial value of 3 75 mEq/l to a level of 5 40 mEq/l 7 hours after the hyper tonic saline injection was maintained at this level until 13 hours after saline and then began to fall reaching the low value of 2.40 mEq/l just before abortion None of the amniotic fluid samples was contaminated with blood

Changes in Serum

The results in patients A and B are shown in Tables I and II and in Figs. 2 and 5 An attempt was made to standardise fluid intake in these patients at approximately 100 ml./hour

Serum sodium showed a biphasic pattern in patient A for it reached a first peak of 160 mEq/l. only 2 hours after the saline injection was maintained at this level during the third hour

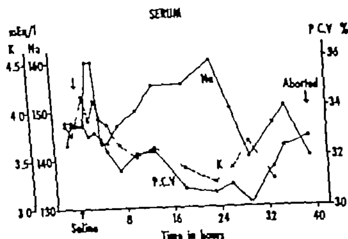


Fig. 2 Patient A. Changes in serum following intra-amniotic injection of hypertonic saline

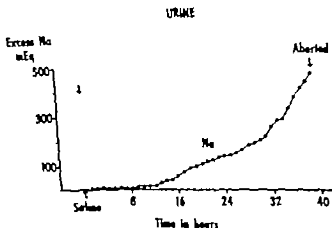


Fig. 3 Patient A. Changes in urine following intra-amniotic injection of hypertonic saline

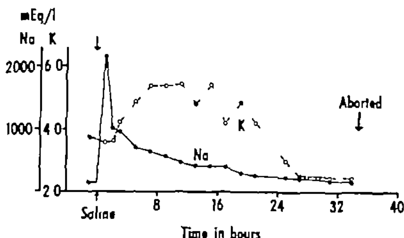


Fig 4 Patient B Changes in amniotic fluid following intra-amniotic injection of hypertonic saline

and then returned to normal after 4 hours It then began to rise more slowly and again reached 160 mEq/l. by 23 hours thereafter gradually falling to normal by the time of abortion Such high sodium levels were not found in patient B and the highest reading—147 mEq/l—was found just before abortion Serum sodium before the saline injection was however lower in B than A and the overall rise of 10 mEq/l in the second and third hour after saline found in B was of similar magnitude to the initial rise in patient A In B sodium levels of 144 mEq/l. were also found at the twelfth and seventeenth hours and again later in the abortion process being 147 mEq/l 30 minutes before the foetus was expelled

Serum potassium showed similar changes in both patients. From a normal serum concentration of 3 8–3 9 mEq/l. before the saline injection the levels rose slightly to 4 15 mEq/l in each patient within 3 hours of saline and then fell slowly to a level below that of the pre-injection value by the time of abortion

Packed cell volume changes in patient A suggested that the maximum increase in serum volume may have occurred fairly late in the process of abortion—about the thirtieth hour after saline. In patient B the lowest packed cell volume was recorded

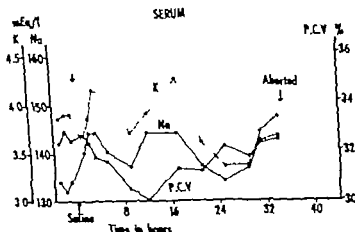


Fig 5. Patient B. Changes in serum following intra-amniotic injection of hypertonic saline.

twelve hours after the saline was injected, suggesting a much earlier rise in serum volume than in patient A.

Serum volume was measured in patients A and B and in 3 other patients by the technique described by Hytten and Paintin (1963) using a 1 per cent aequeous solution of Evans blue dye. Each patient had one estimation of serum volume made before the saline was injected and 3 or 4 further estimations before abortion occurred

All patients showed a rise in serum volume following the intra-amniotic injection of hypertonic saline although the magni tude of the rise varied from 320 ml. to 1340 ml. in different subjects. In patient A the increase measured was 1120 ml. and in patient B 600 ml. The fall in packed cell volume in patients A and B however would be in keeping with a rise in serum volume of 250–300 ml only and the very large increase measured using Evans blue dye must be a gross over-estimate of the true rise.

The marked discrepancy between the fall in packed cell volume and the rise in serum volume may be due to the excess sodium chloride in the maternal circulation altering ionisation of plasma proteins by pH change in the blood. Such a change might result

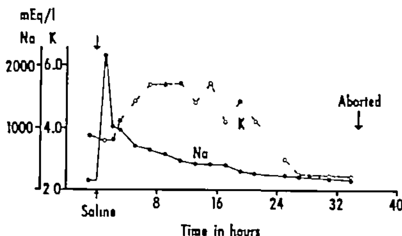


Fig. 4 Patient B. Changes in amniotic fluid following intra amniotic injection of hypertonic saline

and then returned to normal after 4 hours It then began to rise more slowly and again reached 160 mEq/l by 23 hours thereafter gradually falling to normal by the time of abortion Such high sodium levels were not found in patient B and the highest reading—147 mEq/l.—was found just before abortion. Serum sodium before the saline injection was however lower in B than A and the overall rise of 10 mEq/l in the second and third hour after saline found in B was of similar magnitude to the initial rise in patient A. In B sodium levels of 144 mEq/l were also found at the twelfth and seventeenth hours and again later in the abortion process, being 147 mEq/l 30 minutes before the foetus was expelled

Serum potassium showed similar changes in both patients From a normal serum concentration of 3 8–3 9 mEq/l before the saline injection the levels rose slightly to 4 15 mEq/l in each patient within 3 hours of saline and then fell slowly to a level below that of the pre-injection value by the time of abortion.

Packed cell volume changes in patient A suggested that the maximum increase in serum volume may have occurred fairly late in the process of abortion—about the thirtieth hour after saline. In patient B the lowest packed cell volume was recorded

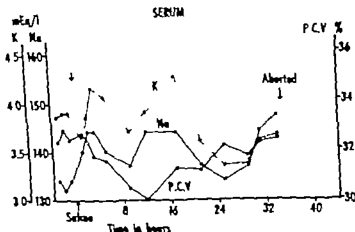


Fig. 5. Patient B. Changes in serum following intra-amniotic injection of hypertonic saline

twelve hours after the saline was injected, suggesting a much earlier rise in serum volume than in patient A.

Serum volume was measured in patients A and B and in 3 other patients by the technique described by Hytten and Paintin (1963) using a 1 per cent aequeous solution of Evans blue dye. Each patient had one estimation of serum volume made before the saline was injected and 3 or 4 further estimations before abortion occurred.

All patients showed a rise in serum volume following the intra-amniotic injection of hypertonic saline although the magnitude of the rise varied from 320 ml. to 1340 ml. in different subjects. In patient A the increase measured was 1120 ml. and in patient B 600 ml. The fall in packed cell volume in patients A and B however would be in keeping with a rise in serum volume of 250–300 ml only and the very large increase measured using Evans blue dye must be a gross over-estimate of the true rise.

The marked discrepancy between the fall in packed cell volume and the rise in serum volume may be due to the excess sodium chloride in the maternal circulation altering ionisation of plasma proteins by pH change in the blood. Such a change might result

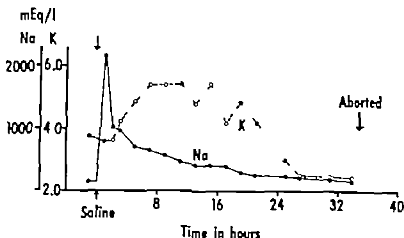


Fig. 4 Patient B. Changes in amniotic fluid following intra-amniotic injection of hypertonic saline.

and then returned to normal after 4 hours It then began to rise more slowly and again reached 160 mEq/l by 23 hours thereafter gradually falling to normal by the time of abortion. Such high sodium levels were not found in patient B and the highest reading—147 mEq/l.—was found just before abortion Serum sodium before the saline injection was however lower in B than A and the overall rise of 10 mEq/l in the second and third hour after saline found in B was of similar magnitude to the initial rise in patient A In B sodium levels of 144 mEq/l were also found at the twelfth and seventeenth hours and again later in the abortion process being 147 mEq/l 30 minutes before the foetus was expelled.

Serum potassium showed similar changes in both patients. From a normal serum concentration of 3 8–3 9 mEq/l. before the saline injection the levels rose slightly to 4 15 mEq/l in each patient within 3 hours of saline and then fell slowly to a level below that of the pre-injection value by the time of abortion.

Packed cell volume changes in patient A suggested that the maximum increase in serum volume may have occurred fairly late in the process of abortion—about the thirtieth hour after saline In patient B the lowest packed cell volume was recorded

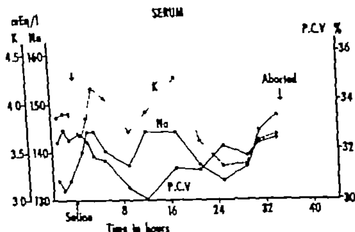


Fig 5 Patient B Changes in serum following intra-amniotic injection of hypertonic saline.

twelve hours after the saline was injected, suggesting a much earlier rise in serum volume than in patient A.

Serum volume was measured in patients A and B and in 3 other patients by the technique described by Hytten and Paintin (1963) using a 1 per cent aequeous solution of Evans blue dye. Each patient had one estimation of serum volume made before the saline was injected and 3 or 4 further estimations before abortion occurred.

All patients showed a rise in serum volume following the intra-amniotic injection of hypertonic saline, although the magnitude of the rise varied from 320 ml. to 1340 ml. in different subjects. In patient A the increase measured was 1120 ml. and in patient B 600 ml. The fall in packed cell volume in patients A and B however would be in keeping with a rise in serum volume of 250–300 ml. only and the very large increase measured using Evans blue dye must be a gross over-estimate of the true rise.

The marked discrepancy between the fall in packed cell volume and the rise in serum volume may be due to the excess sodium chloride in the maternal circulation altering ionisation of plasma proteins by pH change in the blood. Such a change might result

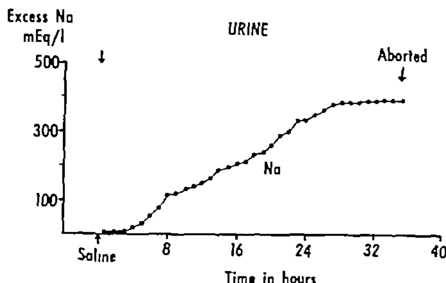


Fig. 6. Patient B. Changes in urine following intra-amniotic injection of hypertonic saline.

in less Evans blue dye than normal being adsorbed onto the plasma albumin and hence account for the false high serum volume results

Serum osmolality results in patients A and B are shown in Tables I and II. Before saline a value of approximately 280 m.osmols./kg. was found—normal for mid-pregnancy Serum osmolality quickly rose after the saline was injected, reaching its highest level of 295–296 m.osmols./kg. 5 hours later Twenty-four hours after saline it had fallen to a level lower than the preinjection value and continued to fall so that by the time of abortion values of 274–276 m osmols /kg were reached.

Changes in Urine Antidiuretic Response

The rise in serum sodium and osmolality within the first few hours of the intra-amniotic injection of hypertonic saline seemed likely to induce release of antidiuretic hormone. To test this hypothesis the effect of the intra-amniotic hypertonic saline injection on urinary output was measured after diuresis had been induced. The antidiuretic effect of the saline was compared with that produced by a known intravenous dose of vasopressin.

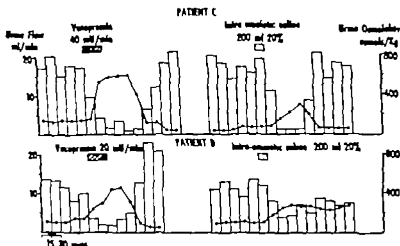


Fig 7 Patients C and D The effect of intravenous vasopressin and intra-amniotic injection of hypertonic saline on urine flow and osmolality

Two patients (C and D) were studied. Table III gives the results, shown in graphic form in Fig 7 The marked antidiuresis and concomitant rise in urine osmolality in response to intravenous vasopressin is obvious. Vasopressin was infused at 40 mU/min for 30 minutes in patient C producing an antidiuresis for about 2 hours urine osmolality rising to over 600 m.osmols./kg. In patient D 30 mU/min. was given for 30 minutes with an antidiuresis lasting for about 90 minutes and a maximum urine osmolality of 453 m.osmols./kg.

Patient C produced a more marked although less persistent antidiuretic response to the intra-amniotic injection of hyper tonic saline than patient D In patient C antidiuresis became apparent 30 minutes after the completion of the saline injection and persisted for 75 minutes osmolality was increased to 304 m osmols./kg In patient D the antidiuresis began 30 minutes after the saline had been injected but was less marked although of greater duration than in patient C. The antidiuretic action of the saline was still in evidence 2 ½ hours after the injection with a persistently high urine osmolality (maximum 278 m.osmols./kg.) The patient had by this time retained about a litre

Table III *The Effect of Intravenous Vasopressin and Intra-amniotic Injection of Hypertonic Saline on Urine Flow and Osmolality During Constant Fluid Intake*

Patient C	Urine Flow[1] ml/min.	Urine Osmolality m.osmols./kg	Patient D	Urine Flow ml/min.	Urine Osmolality m.osmols./kg
	16.7	142		13 6	118
	20.0	130		13.3	118
	15 0	140		11.5	115
	17 3	139		7.9	146
	17 0	132		10.0	137
I V Vasopressin 40 mU/min.	9 3	150	I.V Vasopressin 20 mU/min.	3.5	228
	3 7	528		1.5	296
	1 5	575		1.5	442
	3 3	603		3 7	453
	1.3	601		4 9	313
	1 7	405		12.7	104
	6 5	115		23.2	49
	11 7	114		20.8	36
	17 7	50			
	21 3	47			

		19.7	40			11.1	103
		17.7	34			13.2	115
		14.0	38			10.9	101
		17.3	75			9.2	112
Intra-amniotic Hypertonic saline 200 ml 20 %	5	15.7	83	Intra-amniotic Hypertonic-saline 200 ml 20 %	5	13.5	93
		19.0	66			11.7	105
		11.0	81			8.0	183
		1.3	161			4.1	250
		1.3	222			4.2	278
		1.3	304			6.9	271
		8.5	189			4.9	274
		20.7	64			8.7	244
		14.0	56			7.9	237
		17.7	74			7.3	260
		17.3	54			7.5	268

Urine flow measured over successive 15-minute periods.

Table III. *The Effect of Intravenous Vasopressin and Intra-amniotic Injection of Hypertonic Saline on Urine Flow and Osmolality During Constant Fluid Intake*

Patient C	Urine Flow ml/min	Urine Osmolality m.osmols./kg	Patient D	Urine Flow ml/min.	Urine Osmolality m.osmols./kg
	16 7	142		13.6	118
	20 0	130		13.3	118
	15 0	140		11.5	115
	17.3	139		7.9	146
	17 0	132		10 0	137
LV Vasopressin 40 mU/min.	9 3	150	LV Vasopressin 20 mU/min	3.5	228
	3 7	528		1.5	296
	1.5	575		1.5	442
	3.3	603		3 7	453
	1 3	601		4.9	313
	1 7	405		12.7	104
	6 5	115		23.2	49
	11 7	114		20.8	36
	17 7	50			
	21.3	47			

	19 7	40			11 1	103
	17 7	34			13 2	115
	14 0	36			10 9	101
	17 3	75			9 2	112
Intra-amniotic Hypertonic saline 200 ml 20 %	15 7	63		Intra-amniotic Hypertonic-saline 200 ml 20 %	13 5	93
	19 0	66			11 7	105
	11 0	81			8 0	183
	1 3	161			4 1	250
	1 3	222			4 2	278
	1 3	304			6 9	271
	8 5	189			4 9	274
	20 7	64			8 7	244
	14 0	56			7 9	237
	17 7	74			7 3	260
	17 3	54			7 5	268

Urine flow measured over successive 15-minute periods.

of fluid and was feeling "shivery" the infusion was discontinued and no subsequent ill effects were observed.

It is difficult to quantitate the antidiuretic response to intra-amniotic saline in terms of vasopressin secretion since the response differed in the patients studied. From the data presented it would seem that the antidiuretic response to the hypertonic saline injection would be equivalent to vasopressin secretion of rather less than 20 mU/min.

Urinary sodium and potassium excretion in patients A and B was measured in hourly urine samples the bladder being completely emptied each hour through the indwelling catheter and the urine volume measured. Fluid intake was limited to approximately 100 ml./hour as previously described. The total excretion of sodium and potassium per hour was calculated and the mean excretion of each electrolyte per hour before the saline was injected was subtracted from the total hourly excretion in each urine sample after the saline injection. In this way the "excess" sodium and potassium excreted each hour after saline was estimated and Tables I and II and Figs. 3 and 6 show these values as hourly cumulative totals in milli-equivalents.

Patient A took 20 hours to excrete 100 milli-equivalents of excess urinary sodium, whereas patient B took only 8 hours to excrete the same amount. This may be because patient A had a more marked antidiuresis in response to saline than patient B. Later patient A excreted 270 milli-equivalents of excess sodium in the 10 hours before abortion, whereas patient B excreted only 40 milli-equivalents during a similar period. At the time of abortion patient A had excreted 468 milli-equivalents of extra sodium (i.e. 69 per cent of the 683 milli-equivalents of sodium injected into the amniotic sac). Comparable figures for patient B were 390 milli-equivalents or 57 per cent of the sodium injected. Abortion occurred 4 hours earlier in patient B than in A and urine volumes were lower in the last few hours in B.

As expected, very little excess potassium was excreted in the urine by the time of abortion 15.8 milli-equivalents in patient A and 28.8 milli-equivalents in patient B.

Urine osmolality in general, rose by approximately 200 m. osmols./kg. and high values persisted for almost the whole dura-

tion of abortion although in the last few hours they were tending to fall to more normal levels.

Discussion

Our observations show that when hypertonic saline is injected into the amniotic sac, the subsequent effect on the maternal body fluids is much greater than might be expected from clinical observation of the patient. Apart from a fairly intense thirst which in most patients comes on about one hour after the saline injection and persists for several hours, a normal, healthy woman experiences no obvious upset.

We have shown that the sodium concentration in the amniotic fluid falls rapidly after the saline is injected reaching 25 per cent of its initial concentration after 12 hours. As amniotic fluid sodium falls, serum sodium and osmolality rise, attaining their maximum values only a few hours after the saline injection. In response to this a temporary antidiuresis is induced. As serum sodium and osmolality fall, the excess sodium begins to be excreted in the urine so that by the time of abortion 60 to 70 per cent of the sodium injected has been excreted. Taking into account the excess sodium remaining in the amniotic fluid at abortion, most of the injected sodium can be accounted for. We have recently measured chloride concentration in amniotic fluid and urine using the Eel Chloride Meter and have found an almost identical pattern to sodium suggesting that most of the sodium chloride injected into the uterus enters the blood stream and is subsequently excreted in the urine.

The falling sodium concentration could, of course, be explained by an increasing volume of amniotic fluid. Using inulin, *Wagner* (1966) has described a 25 to 30 per cent increase in amniotic fluid volume during the first three hours after hypertonic saline and *Csapo et al* (1963) suggested that hypertonic solutions may initiate labour by an increase in uterine volume. Increasing amniotic fluid volume could therefore partly account for the reduction in sodium concentration but cannot be the complete explanation since so much of the injected sodium chloride can be accounted for in the urine.

King Friedman and *Steer* (1964) found that potassium levels in the amniotic fluid fell for 14 hours after the saline was in jected and then rose and suggested that this was a dilution effect due to increasing volume We have found a very different pattern of potassium concentration in the amniotic fluid with rising levels for approximately 14 hours and then a marked fall. The foetus remained alive in the case studied by *King, Friedman* and *Steer* whereas in our patient it is likely that the foetus succumbed soon after the saline injection. Partial disintegration of the dead foetus may have led to the initial rise in potassium, its subsequent loss from the amniotic fluid and excretion in the urine being shown by the very low potassium concentration in the amniotic fluid by the time of abortion.

Bengtsson and *Csapo* (1962) claimed that pre-labour uterine contractions began only when the amniotic fluid was no longer hypertonic 16 to 22 hours after saline injection and took this as evidence that the "defence mechanism had to be suppressed before uterine activity could evolve We have never found the amniotic fluid to regain its isotonicity before abortion and have shown (*Turnbull and Anderson* 1965) that uterine activity first begins to increase 40 to 80 minutes after the hypertonic saline injection, the intensity of contractions steadily increasing until abortion. It is of interest that the uterus starts to contract at a time when serum sodium is reaching a peak, and when vasopres sin is presumably being secreted. *Hendricks et al* (1959) showed increased uterine activity following intravenous infusion of 2.5 or 5 per cent saline solution in 9 of 14 patients in late pregnancy

The variation in the response of the body fluids to the intra amniotic injection of hypertonic saline may be related to the speed at which the saline leaves the amniotic fluid compartment and is excreted in the urine We were recently informed of a patient with mitral stenosis who developed pulmonary oedema on the third post-partum day following therapeutic abortion in duced by the intra-amniotic injection of hypertonic saline One of our own patients with no cardiac or renal disease developed marked ankle oedema four days after saline injection. Neither of these patients had oedema during the abortion process and it is probable that in both, retention of the excess sodium had

occurred leading to clinical signs of oedema several days after the saline injection.

The potential dangers of a rise in serum volume and sodium after intra-amniotic hypertonic saline in the patient with poor cardiac or renal function and in pre-eclamptic toxaemia have already been pointed out (*Turnbull* and *Anderson* 1966). Although we now postulate the rise in serum volume to be a modest one—possibly only 250 to 300 ml in the two patients investigated—any sudden increase in circulating blood volume might lead to cardiac failure in a patient with severe cardiac disease, and the magnitude of the rise may depend on the rate of influx of electrolytes into the maternal circulation.

In previous studies it has been shown that following the intra-amniotic injection of hypertonic saline the urinary output of pregnanediol remains at nearly normal levels until placental separation occurs (*Klopper*, *Turnbull* and *Anderson* 1966) and histological techniques demonstrated that at least 80 per cent of the placenta is undamaged and apparently continuing its endocrine function (*Christie*, *Anderson*, *Turnbull* and *Beck* 1966). These findings make it unlikely that the effectiveness of hypertonic saline in inducing uterine contractions is due to the removal of a progesterone "block" on the myometrium as a result of extensive placental damage.

The present study has shown that, with the influx of sodium chloride from the amniotic fluid into the maternal circulation, movement of electrolytes and water must occur between extracellular and intra-cellular compartments. Such changes in and around the myometrial cell might well alter its membrane potential and lead to the onset of uterine contractions. Measurement of intra- and extra-cellular electrolytes however must await the development of accurate techniques, when perhaps the controlling mechanism of the onset of labour following intra-amniotic injection of hypertonic saline will be elucidated.

SUMMARY

A systematic study has been made of the sequential changes in amniotic fluid and in maternal body fluids following the intra-amniotic injection of hypertonic saline in mid-pregnancy.

King Friedman and *Steer* (1964) found that potassium levels in the amniotic fluid fell for 14 hours after the saline was in jected and then rose and suggested that this was a dilution effect due to increasing volume We have found a very different pattern of potassium concentration in the amniotic fluid with rising levels for approximately 14 hours and then a marked fall. The foetus remained alive in the case studied by *King, Friedman* and *Steer* whereas in our patient it is likely that the foetus succumbed soon after the saline injection. Partial disintegration of the dead foetus may have led to the initial rise in potassium, its subsequent loss from the amniotic fluid and excretion in the urine being shown by the very low potassium concentration in the amniotic fluid by the time of abortion

Bengtsson and *Csapo* (1962) claimed that pre-labour uterine contractions began only when the amniotic fluid was no longer hypertonic, 16 to 22 hours after saline injection and took this as evidence that the defence mechanism had to be suppressed before uterine activity could evolve We have never found the amniotic fluid to regain its isotonicity before abortion, and have shown (*Turnbull* and *Anderson* 1965) that uterine activity first begins to increase 40 to 80 minutes after the hypertonic saline injection the intensity of contractions steadily increasing until abortion. It is of interest that the uterus starts to contract at a time when serum sodium is reaching a peak and when vasopres sin is presumably being secreted *Hendricks et al* (1959) showed increased uterine activity following intravenous infusion of 2.5 or 5 per cent saline solution in 9 of 14 patients in late pregnancy

The variation in the response of the body fluids to the intra amniotic injection of hypertonic saline may be related to the speed at which the saline leaves the amniotic fluid compartment and is excreted in the urine We were recently informed of a patient with mitral stenosis who developed pulmonary oedema on the third post partum day following therapeutic abortion in duced by the intra-amniotic injection of hypertonic saline. One of our own patients with no cardiac or renal disease developed marked ankle oedema four days after saline injection Neither of these patients had oedema during the abortion process and it is probable that in both, retention of the excess sodium had

occurred leading to clinical signs of oedema several days after the saline injection.

The potential dangers of a rise in serum volume and sodium after intra-amniotic hypertonic saline in the patient with poor cardiac or renal function and in pre-eclamptic toxaemia have already been pointed out (*Turnbull* and *Anderson* 1966). Although we now postulate the rise in serum volume to be a modest one—possibly only 250 to 300 ml. in the two patients investigated—any sudden increase in circulating blood volume might lead to cardiac failure in a patient with severe cardiac disease, and the magnitude of the rise may depend on the rate of influx of electrolytes into the maternal circulation.

In previous studies it has been shown that following the intra-amniotic injection of hypertonic saline the urinary output of pregnanediol remains at nearly normal levels until placental separation occurs (*Klopper Turnbull* and *Anderson* 1966) and histological techniques demonstrated that at least 80 per cent of the placenta is undamaged and apparently continuing its endocrine function (*Christie Anderson Turnbull* and *Beck* 1966). These findings make it unlikely that the effectiveness of hypertonic saline in inducing uterine contractions is due to the removal of a progesterone "block" on the myometrium as a result of extensive placental damage.

The present study has shown that, with the influx of sodium chloride from the amniotic fluid into the maternal circulation, movement of electrolytes and water must occur between extracellular and intra-cellular compartments. Such changes in and around the myometrial cell might well alter its membrane potential and lead to the onset of uterine contractions. Measurement of intra- and extra-cellular electrolytes, however, must await the development of accurate techniques, when perhaps the controlling mechanism of the onset of labour following intra-amniotic injection of hypertonic saline will be elucidated.

SUMMARY

A systematic study has been made of the sequential changes in amniotic fluid and in maternal body fluids following the intra-amniotic injection of hypertonic saline in mid-pregnancy.

In amniotic fluid sodium concentration after the saline injection was greater than 2000 mEq/l. and although this fell rapidly during the next 12 hours it had not returned to pre-injection levels at the time of abortion potassium concentration rose for several hours but fell to low levels in the late stages of the abortion process. The sodium concentration and osmolality of the serum rose to a maximum a few hours after the saline injection, inducing a temporary antidiuresis Serum volume increased by 250 to 300 ml. By the time of abortion 60 to 70 per cent of the sodium injected into the amniotic fluid had been excreted in the urine.

The potential hazards of the technique in patients with poor cardiac or renal function are discussed.

Acknowledgements

This work was carried out during the tenure of a grant from the Medical Research Council. We are grateful to the laboratory staff of the Clinical Research Unit, Aberdeen Maternity Hospital for valuable technical assistance.

REFERENCES

Bengtsson L. Ph. and *Csapo A. I* Amer J Obstet. Gynec 83 1083 1962
Cameron J M and *Dayan A. D* Brit. med. J *i* 1010 1966
Christie J Anderson A. B. M. Turnbull A. C and *Beck J S* J Obstet. Gynaec. Brit. Cwlth., 73 399 1966
Csapo A. I Jaffi H Kerenyi T de Mattos C E R. and *de Sousa Filho M B* Amer J Obstet. Gynec 87 892, 1963
Hendricks C H Helfand T and *Caldeyro-Barcia R* Amer J Obstet. Gynec., 77 387 1959
Hytten F E and *Paintin D B* J Obstet Gynaec Brit. Cwlth 70 40., 1963
King, T M Friedman J and *Steer C M* Bull Sloan Hosp. Women, *I* 14 1964
Klopper A. Turnbull A. C. and *Anderson A. B M* J Obstet Gynaec Brit. Cwlth., 73 390, 1966
Pinkerton J H M Brit. med J *i* 1049 1966
Turnbull A. C and *Anderson A. B M* J Obstet Gynaec. Brit. Cwlth., 72 755 1965
– Brit. med. J *i* 672, 1966
Wagatsuma T Amer J Obstet Gynec., 93 743, 1965

Wagner G Clin. Obstet. Gynec., *9* 520 1966
Weingold A. B Seigel S and Stone M L., Obstet. Gynec., *26* 622, 1965
Wiqvist N and Eriksson G Amer J. Obstet. Gynec., *88* 75, 1964

Received on March 30 1967

Author present address,
Department of Obstetrics, Welsh
National School of Medicine,
The Maternity Hospital, Glossop
Terrace CARDIFF Wales, U.K.

In amniotic fluid, sodium concentration after the saline injection was greater than 2000 mEq/l and although this fell rapidly during the next 12 hours it had not returned to pre-injection levels at the time of abortion potassium concentration rose for several hours but fell to low levels in the late stages of the abortion process. The sodium concentration and osmolality of the serum rose to a maximum a few hours after the saline injection, inducing a temporary antidiuresis Serum volume increased by 250 to 300 ml. By the time of abortion 60 to 70 per cent of the sodium injected into the amniotic fluid had been excreted in the urine.

The potential hazards of the technique in patients with poor cardiac or renal function are discussed.

Acknowledgements

This work was carried out during the tenure of a grant from the Medical Research Council. We are grateful to the laboratory staff of the Clinical Research Unit, Aberdeen Maternity Hospital, for valuable technical assistance

REFERENCES

Bengtsson L. *Ph.* and *Csapo* A. I Amer J Obstet. Gynec., 83 1083 1962
Cameron J M and *Dayan* A. D Brit. med. J., *1* 1010 1966
Christie J *Anderson* A. B. M. *Turnbull* A. C. and *Beck* J S J Obstet. Gynaec. Brit. Cwlth., 73 399 1966
Csapo A. I *Jaffin* H *Kerenyi* T *de Mattos* C E. R. and *de Sousa Filho* M B. Amer J Obstet Gynec 87 89_, 1963
Hendricks C. H *Helfand* T and *Caldeyro-Barcia* R. Amer J Obstet. Gynec., 77 387 1959
Hytten F E and *Paintin* D B J Obstet. Gynaec. Brit. Cwlth 70 402, 1963
King, T M *Friedman* J and *Steer* C. M Bull. Sloan Hosp Women, I 14 1964
Klopper A. *Turnbull* A. C and *Anderson* A. B. M J Obstet Gynaec Brit. Cwlth., 73 390 1966
Pickerton J H M Brit. med. J *1* 1049 1966
Turnbull A. C. and *Anderson* A. B. M., J Obstet. Gynaec. Brit Cwlth 2 755 1965
– Brit. med. J *1* 672, 1966
Wagatsuma, T., Amer J Obstet Gynec., 93 743 1965

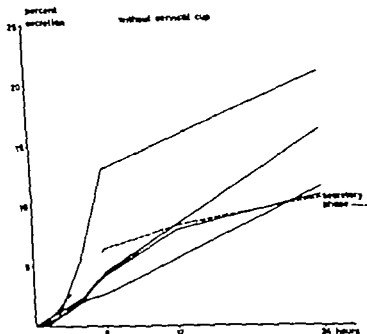


Fig. 1. The cumulative urinary excretion of radio activity after vaginal deposition of tritium labelled prostaglandin E_1. 3 women in the proliferatory phase and 1 woman in the secretory phase.

Material and Method

The investigation was performed on normally menstruating volunteers without any signs of infection. Tritium labelled prostaglandin E (PGE_1 5 6-^{3}H 6 10^7 dpm) prepared and kindly supplied by professor B. Samuelsson, Kungl. Veterinärhögskolan, Stockholm, was dissolved in 2 drops of ethanol and diluted with 2 ml of normal seminal plasma. This solution was deposited in the posterior fornix through a thin polyethylene catheter attached to a syringe and this was followed by closure of the vulva with adhesive tape. After this procedure the volunteers were left in the Trendelenburg position for six hours.

Before the administration of the labelled PGE_1 a catheter connected to a bag was introduced into the bladder for the collection

Sandberg, F Ingelman-Sundberg, A Rydén G and Joelsson, I Acta obst. et gynec. scandinav 47 22, 1968

From the Department of Obstetrics and Gynaecology (Professor Axel Ingelman-Sundberg) Sabbatsberg Hospital Kungl Karolinska Institutet Stockholm and the Department of Pharmacognosy (Professor Finn Sandberg) Kungl Farmaceutiska Institutet Stockholm Sweden

THE ABSORPTION OF TRITIUM LABELLED PROSTAGLANDIN E_1 FROM THE VAGINA OF NON PREGNANT WOMEN

BY

F SANDBERG A. INGELMAN SUNDBERG G RYDÉN AND I. JOELSSON

In previous papers (*Sandberg et al* 1963 1964 1965) the effects of different prostaglandins on the Fallopian tubes and the uterus of non pregnant women was studied *in vitro* with the Magnus-Kehrer technique. The most interesting effect was found with prostaglandin E_1 (PGE_1) and prostaglandin E_2 (PGE_2) They exerted a specific action on the longitudinal musculature of the tube consisting of contraction of the proximal 1/4 and relaxation of the rest of the tube This effect might be of special importance for the fertilization of the ovum.

Already in 1947 *Asplund* after vaginal instillation of a prostaglandin extract from human semen in rabbits observed a decrease in blood pressure indicating absorption of the prostaglandin through the vaginal wall and *Eliasson* and *Posse* (1965) using Rubin s Test found an increase in the resistance to insufflation at midcycle in 3 out of 7 infertile women with patent tubes, following the vaginal deposition of a purified prostaglandin extract in amounts corresponding to a normal ejaculate

As a normal human ejaculate contains PGE_1 and PGE_2 in amounts of 25 μg/ml and 23 μg/ml respectively (*Bygdeman* and *Samuelsson* 1966) it was considered of interest to study the vaginal absorption of these substances quantitatively

From a theoretical point of view the absorption of PGE_1 and PGE_2 through the vaginal wall can be postulated to be similar Since only tritium labelled PGE_1 was available our investigations were confined to this substance

50 mg in 1000 ml of toluene). The tritium activity was determined in a Packard Tri-Carb Liquid Scintillation Spectrometer Model 314 AS after at least two days storage of the samples in the apparatus. The counting efficiency (generally about 8–10 per cent) was calculated by the use of an internal standard of tritium toluene. The loss of radio activity in the syringe and catheter was allowed for.

Results

The results obtained are summarized in Figures 1 and 2. It is evident from the figures, that within the time of observation (24–32 hours) 10–25 per cent of the radio activity administered is excreted irrespective of the phase of the menstrual cycle, and whether the external os has been covered or not. As the absorption was interrupted after 6 hours and there is still an excretion of radio activity 24 hours later the elimination of the prostaglandin metabolites through the kidney must be rather slow.

Discussion

After subcutaneous injection of tritium labelled PGE_1 Samuelsson (1964) in experiments on rats, recovered 50 per cent of the radio activity in the urine and 10 per cent in the faeces within 20 hours. As the subcutaneous administration used by Samuelsson corresponds to complete absorption, our recovery rate of 10–25 per cent in the urine may therefore indicate a vaginal absorption of about 20–50 per cent. Since there was no difference in excretion between the volunteers with and without a cervical cup, the absorption through the cervical canal must be of no significance. Any absorption of prostaglandin of physiological importance for the process of fertilization must occur within a few hours of ejaculation. No conclusion can be drawn from the present investigation concerning the absorption rate during this period, since elimination through the kidney is so slow. *Eliasson* and *Posse* (1965) observed an increase in the resistance to insufflation during Rubin's Test within 25–40 minutes indicating a rapid vaginal absorption of prostaglandins in nonpregnant women. However before any conclusions can be drawn concerning the physiological

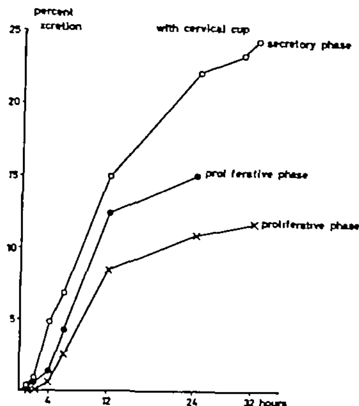


Fig 2. The cumulative urinary excretion of radio activity after vaginal deposition of tritium labelled prostaglandin E_1 in 3 women with a cervical cap fitted over the external os.

of the urine at certain intervals In the first series the urine was collected after 1 2, 4 6 12 and 24 hours and in the second series after 1 2, 4 6 12 24 and 32 hours. In the latter series a cervical cup was fitted over the cervix before the administration of PGE_1 *After 6 hours of exposure the vagina was washed with 0 1 %* benzalkonium chloride solution

The extraction of labelled PGE_1 and its metabolites was performed as follows 25 ml of urine was shaken 3 times with 50 ml of water saturated n butanol The mixture was acidified with 2 N HCl to pH 3 0 The butanol phase was washed three times with 15 ml of distilled water evaporated to dryness and dissolved in 3 ml of methanol 0.5 ml of this methanolic solution was mixed with 15 ml of a scintillator solution (PPO 5 g, *dimethyl* POPOP

Koch, G and Wendel, H Acta obst et gynec scandinav 47 27 1968
From the Departments of Clinical Physiology (Prof H Linderholm) and Pediatrics (Prof S Sjölin) University of Umeå Sweden

THE EFFECT OF PETHIDINE ON THE POSTNATAL ADJUSTMENT OF RESPIRATION AND ACID BASE BALANCE

BY

G. KOCH AND H. WENDEL

Pethidine (synonyms demerol, meperidine dolantin) is a synthetic compound that resembles morphine in its analgesic properties and atropine in its antispasmodic effects (*Carmody* 1963) In labour it not only relieves pain but is believed to exert a relaxing effect on the cervix (*Carmody* 1963) and a regulating effect on abnormal uterine action (*Lindgren* 1966) While doses between 100 and 400 mg noticeably shorten labour in primiparas (Gilbert and Dixon 1943) very high doses exceeding 1000 mg appear to induce inertia thus prolonging labour (*James* 1960) and increasing the incidence of instrumental deliveries and Caesarean section (*Gordon* and *Pinker* 1958 *James* 1960)

Pethidine has been shown to cross the placental barrier The rate and degree of this transfer appears to depend on the dose and the time of administration (*Apgar Burns Brodie* and *Papper* 1952 *Crawford* and *Rudofsky* 1965) The great advantage of pethidine over morphine has been claimed to be its less depressant effect on the newborn infant's respiration (Gilbert and *Dixon* 1943) and morphine has actually been shown to decrease the effecti e ventilation, in response to CO inhalation more than pethidine when used in equivalent doses in the *newborn infant* (*Way Costley* and *Way* 1965)

Several authors report no or only negligible respiratory depression of the newborn infant when pethidine is given to the mother (*Apgar Burns Brodie* and *Papper* 1952 *Gilbert* and

importance of the prostaglandin content of the ejaculate additional similar experiments must be performed in normally fertile women

SUMMARY

Tritium labelled prostaglandin E_1 was deposited for 6 hours in the posterior fornix of 7 normally menstruating volunteers. 10–25 per cent of the radio activity administered was recovered in the urine within 24–32 hours. The application of a cervical cup to the cervix did not influence the absorption which is roughly estimated to be 20–50 per cent. The physiological significance of this absorption is still uncertain

Acknowledgements

We are indebted to professor Sune Bergström Karolinska institutet and to professor Bengt Samuelsson Kungl Veterinärhögskolan for kindly supplying the tritium labelled prostaglandin E_1 This work was supported by the Swedish Medical Research Council

REFERENCES

Asplund J Acta Physiol. Scand. *13* 109 1947
Bygdeman M and *Samuelsson B.* Clin. Chim. Acta *13* 465 1966
Eliasson R. and *Posse N* Acta obst. et gynec. scandinav 39 112, 1960
– *Ibid.* Int. J Fertil *10* 373 1965
Samuelsson B Proc Sec. Int Congr Endocrin. London 1964 p 84 –856
Sandberg, F Ingelman-Sundberg, A. and *Rydén G* Acta obst. et gynec. scandinav *42* 269 1963
– *Ibid. 43* 95 1964
– *Ibid. 44* 585 1965

Received on May 5 1967

Koch, G and Wendel, H Acta obst et gynec scandinav 47 27 1968
From the Departments of Clinical Physiology (Prof H Linderholm) and Pediatrics (Prof S Sjölin) University of Umeå Sweden

THE EFFECT OF PETHIDINE ON THE POSTNATAL ADJUSTMENT OF RESPIRATION AND ACID BASE BALANCE

BY

G. KOCH AND H. WENDEL

Pethidine (synonyms demerol meperidine dolantin) is a synthetic compound that resembles morphine in its analgesic properties and atropine in its antispasmodic effects (*Carmody* 1963) In labour it not only relieves pain but is believed to exert a relaxing effect on the cervix (*Carmody* 1963) and a regulating effect on abnormal uterine action (*Lindgren* 1966) While doses between 100 and 400 mg noticeably shorten labour in primiparas (*Gilbert* and *Dixon*, 1943) very high doses exceeding 1000 mg appear to induce inertia thus prolonging labour (*James* 1960) and increasing the incidence of instrumental deliveries and Caesarean section (*Gordon* and *Pinker* 1958 *James* 1960)

Pethidine has been shown to cross the placental barrier The rate and degree of this transfer appears to depend on the dose and the time of administration (*Apgar Burns Brodie* and *Papper* 1952 *Crawford* and *Rudofsky* 1965) The great advantage of pethidine over morphine has been claimed to be its less depressant effect on the newborn infant s respiration (*Gilbert* and *Dixon* 1943) and morphine has actually been shown to decrease the effective ventilation, in response to CO inhalation, more than pethidine when used in equivalent doses, in the newborn infant (*Way Costley* and *Way* 1965)

Several authors report no or only negligible, respiratory depression of the newborn infant when pethidine is given to the mother (*Apgar Burns Brodie* and *Papper* 1952 *Gilbert* and

importance of the prostaglandin content of the ejaculate additional similar experiments must be performed in normally fertile women

SUMMARY

Tritium labelled prostaglandin E_1 was deposited for 6 hours in the posterior fornix of 7 normally menstruating volunteers. 10–25 per cent of the radio activity administered was recovered in the urine within 24–32 hours. The application of a cervical cup to the cervix did not influence the absorption which is roughly estimated to be 20–50 per cent. The physiological significance of this absorption is still uncertain

Acknowledgements

We are indebted to professor Sune Bergström Karolinska Institutet and to professor Bengt Samuelsson Kungl. Veterinärhögskolan for kindly supplying the tritium labelled prostaglandin E_1 This work was supported by the Swedish Medical Research Council

REFERENCES

Asplund J Acta Physiol. Scand. *13* 109 1947
Bygdeman M and *Samuelsson B* Clin Chim. Acta *13* 465 1966
Eliasson R. and *Posse N* Acta obst. et gynec. scandinav 39 112, 1960
– *Ibid.* Int. J Fertil. *10* 373 1965
Samuelsson B Proc Sec Int. Congr Endocrin London 1964 p 847–856
Sandberg, F Ingelman-Sundberg, A. and *Rydén G* Acta obst. et gynec. scandinav *42* 269 1963
– *Ibid. 43* 95 1964
– *Ibid. 44* 585 1965

Received on May 5 1967

Table I. Some Relevant Obstetrical Data

No Sex	Age of Mother (Years)	Parity	Length of Pregnancy (Weeks)	Duration of Labour[1] (Hours)	Pethidine: Time of Adm. Before Delivery (Hours)	Pethidine: Dosage	Anaesthesia: Nitrous oxide[2]	Anaesthesia: Trichlorethylene	Birth Weight (Grams)	Length at Birth (cm)
idine Group										
M	31	IV	40	4.9	0.9	100	+	–	3090	50
F	22	I	42	16.3	3.3	100	+	+	4040	53
F	21	I	38	6.8	2.3	100	–	+	2720	48
F	29	II	41	2.2	1.2	50	+	–	3150	48.5
F	19	I	41	9.6	1.8	100	+	+	3270	50
M	25	I	42	7.7	3.5	100	+	–	3490	54
M	17	I	41	4.2	2.5	100	+	+	4260	54
F	23	II	40	2.3	1.8	100	–	+	3180	52
ean	23.4		41	4.5	2.2		6/8	5/8	3400	51
Control Group										
1 F	30	IV	40	–			–	–	3020	49
2 F	35	III	42	–			+	+	3330	51
3 F	22	I	43	17.0			+	+	3500	51
4 F	25	II	41	3.0				+	3970	53.5
5 F	29	II	39	2.3			+	–	3310	52
6 M	34	III	40	3.8			+	+	3570	52
7 F	42	III	43	4.3				+	3560	51
8 F	19	I	42	3.6			+	+	3400	52
9 F	32	III	41	2.0				–	4900	53
10 F	20	I	42	–				+	3200	48
11 M	18	I	41				+	–	3600	51
12 M	25	II	43	2.8				+	3220	52
Mean	27.7		41	4.8			10/12	8/12	3548	51.5

[1] Duration of labour is the time elapsing from cervical dilatation of 2 centimeters to delivery

[2] Nitrous oxide was at that time given as pure nitrous oxide (without oxygen

the two groups is that the mothers in the first group (n = 8) received pethidine within 3 ½ hours of delivery while those in the second group had no premedication (n = 12)

All the infants were normal and took part in a systematic study of the postnatal adjustment of blood gases and acid base balance

Dixon 1943 *Potts* and *Ullery* 1961 *Roby* and *Schumann* 1943 *Ullery* and *Bair* 1962 *Winters Garcia* and *Lubin* 1951) while others consider pethidine to have a depressant effect (*Carmody* 1963 *Paterson* and *Prescott* 1954 *Stephen Nowill* and *Martin* 1952) Even postnatal death has been reported (*Amias* and *Fairbairn* 1963) in which pethidine was thought to be a determining factor

In most reports however evaluation of the condition of the newborn infant was subjective, and it has not been until the last few years that a more objective approach has been attempted for estimating the effects on the baby of drugs used for obstetric analgesia Thus there have been reports both of a statistically significant reduction in the respiratory minute volume of infants born after administration of pethidine to the mother (*Roberts Kane Percival* and *Please* 1957) and also of an initial depression in the blood oxygen saturation of the normal full term infant delivered after obstetrical premedication of the mother with pethidine and scopolamine (*Taylor Fumetti Essig, Goodman* and *Walker* 1955) On the other hand, pethidine used in combination with promethazine could not be shown to have caused depression when newborn infants were studied by determining their blood oxygen saturation (*Potts* and *Ullery* 1961) There seems to be only one report (*James* 1960) concerning ventilation in terms of Pa_{CO_2} and acid base balance Since pethidine was used in combination with other drugs in this series however no conclusions can be drawn regarding the effect of pethidine alone.

Opinions about the influence of pethidine on the baby thus seem divergent and knowledge about its respiratory effects in the newborn infant is incomplete The purpose of this study was therefore to contribute to a better understanding of those respiratory effects by evaluating them objectively It is a study of the postnatal adjustment of ventilation and of intrapulmonary gas exchange as reflected by arterial blood gases and acid base balance and of their alterations during the first 24 hours of life

Material

Two groups of infants were studied Some relevant obstetrical data are given in Table I The only significant difference between

While there is a slight, but not statistically significant difference, between the two groups with regard to umbilical venous blood composition, pH in arterial blood is markedly lower and Pa_{CO_2} markedly higher in the pethidine group at 10 30 and 60 minutes, and Pa_{CO_2} higher even at 5 hours of age. These differences are statistically significant. pH in the pethidine group is lower even at 5 and 24 hours and Pa_{CO_2} higher at 24 hours but the differences are slight and not statistically significant.

Differences in standard bicarbonate base deficit, lactic acid concentration, Pa_{O_2} during air as well as during breathing 100 per cent oxygen, and calculated anatomic R–L shunt are slight and not statistically significant.

Discussion

There is more pronounced carbon dioxide retention and acidosis during the first minutes and hours of extrauterine life in infants whose mothers have received pethidine within 3½ hours of delivery when compared with a group without pethidine premedication. Judging from standard bicarbonate and base deficit, the increased acidosis is due solely to relatively increased CO_2-retention, i.e. it is purely respiratory in origin. In agreement with the response of the metabolic parameters, standard bicarbonate and base excess, there is no significant difference in lactate concentration, nor is there a significant difference in the degree of oxygenation seen from oxygen tension. Thus it may be concluded that in spite of inducing relative alveolar hypoventilation, and thus decreasing the effective alveolar oxygen tension, pethidine does not restrict the net oxygen transfer i.e. it does not interfere with either the diffusion or the distribution components of intrapulmonary gas exchange. The adjustment of the ratio of effective ventilation to effective perfusion, is during the neonatal period, the most important factor determining arterial oxygen tension, apart from the degree of venous admixture due to anatomical right-to-left shunt (*Prod'hom Levison Cherry Drorbaugh Hubbell Jr* and Smith 1964 Koch 1968 b)

As seen from the oxygen tension levels during oxygen breath-

in the healthy newborn infant The mothers health during preg nancy the gestational age the progress of labour (as judged from partograms) the clinical onset of respiration (including an Apgar score (*Apgar* 1953) exceeding 8) and the clinical course during the neonatal period were all within normal limits The cord was usually clamped after cessation of pulsations and never before 30 seconds had elapsed after delivery of the baby

When the obstetrical histories of all the patients were re- viewed the use of pethidine in some cases was noted, and these infants were selected to form group 1 The 12 infants of the con trol group were selected from a larger series of normal babies (*Koch* and *Wendel* 1968) as they were born at the same period, often the same day as the pethidine" infants They are fully representative of the larger series

Methods

Umbilical venous blood was obtained by puncture of the un clamped umbilical cord, and arterial blood for subsequent exami nations by means of an indwelling umbilical catheter (*Koch* and *Wendel* 1967) The time schedule for the subsequent studies was 10 30 and 60 minutes and 5 as well as 24 hours of age Blood samples were analyzed for pH P_{CO_2} standard bicarbonate and base deficit (= negative base excess according to *Siggaard Andersen et al* 1960) lactic acid concentration and P_{O_2}. From the age of 55 minutes P_{O_2} was also determined after breathing pure oxygen and the anatomical R–L shunt calculated (*Koch* 1968) However for various technical reasons not all determina tions could be performed in every case

The analytical methods used, including their reproducibility are described elsewhere in detail (*Koch* 1968 a *Koch* and *Wen del* 1967 *Koch* and *Wendel* 1968)

Results

The results are given in Table II, III and IV Both groups evi dently follow the general pattern of postnatal adaptation (*Koch* and *Wendel* 1968) which is characterized by initial CO_2-reten- tion, respiratory and metabolic acidosis and hypoxaemia which all show a gradual decrease.

. bec 1												
n	12	6	12	8	12	7	12	6	11	5	11	8
x̄	18.6	19.3	16.9	16.1	18.5	18.2	19.1	19.1	19.4	20.3	20.7	20.0
range	14.5	18.0	13.5	14.5	14.5	15.0–	16.0	16.8	17.0	19.5–	18.0–	19.0–
	21.5	20.5	19.5	19.5	20.5	20.0	21.5	20.5	21.5	21.5	23.0	21.5
SD	1.8	0.9	1.6	1.8	1.6	1.6	1.7	1.4	1.4	1.2	1.5	1.0
$\bar{x}_C$ $\bar{x}_P$	0.7		0.8		0.3		0.0		0.9–		0.7	
p	>0.3		>0.2		>0.7		>0.9		>0.2		>0.6	
n	12	6	12	7	12	7	12	6	11	5	11	8
x̄	6.9	5.8	9.2	10.0	7.1	7.3	6.2	6.3	5.9	4.5	4.2	4.4
range	3.1	4.5	5.8	5.8–	4.5–	5.0	3.1	3.8	3.8	2.5–	1.1–	3.1–
	12.2	7.8	11.5	12.0	12.2	11.0	10.2	9.5	9.0	6.4	7.8	8.4
SD	2.3	0.4	2.1	2.3	2.1	2.0	2.2	2.1	1.8	1.7	2.0	1.2
$\bar{x}_C$ $\bar{x}_P$	1.1		−0.8		−0.2		0.1		1.4		0.2	
p	>0.2		>0.4		>0.8		>0.9		>0.1		>0.8	
n	12	6	12	7	12	6	12	5	11	5	11	8
x̄	27.7	27.1	49.5	52.0	53.7	59.1	62.0	67.4	73.9	73.2	71.9	69.8
range	15.3–	23.0	31.3–	34.7	32.6–	42.7–	45.9–	55.6–	56.9–	62.9–	54.1–	57.2–
	35.7	31.9	69.8	74.8	73.7	81.8	77.4	82.0	88.4	85.0	92.8	81.7
SD	5.2	3.7	10.4	16.0	11.5	16.0	11.6	11.6	10.8	8.9	11.8	7.5
$\bar{x}_C$ $\bar{x}_P$	0.6		2.5		−5.4		−5.4		0.7		2.3	
p	>0.8		>0.6		>0.4		>0.3		>0.9		>0.6	

e II *Comparison of pH Carbon Dioxide Tension (P_{CO_2}) Standard Bicarbonate Base Deficit (BD) and Oxygen Tension Between Control (C) and Pethidine (P) Group ($\bar{x}$=Mean SD=Standard Deviation p=probability)*

		Umbilical Vein		Umbilical Artery									
				10 min.		30 min		60 min		5 hours		24 hours	
		C	P	C	P	C	P	C	P	C	P	C	P
	n	12	6	12	6	12	8	12	6	11	5	11	8
	$\bar{x}$	7.32	7.33	7.24	7.16	7.32	7.24	7.35	7.29	7.35	7.32	7.[illegible]9	7.37
	range	7.18–7.39	7.27–7.41	7.14–7.30	7.10–7.24	7.23–7.37	7.12–7.32	7.29–7.[illegible]9	7.19–7.34	7.26–7.[illegible]9	7.27–7.36	7.33–7.45	7.34–7.40
	SD	0.06	0.04	0.04	0.05	0.04	0.07	0.04	0.05	0.04	0.04	0.03	0.03
	$\bar{x}_C - \bar{x}_P$	−0.009		0.075		0.084		0.057		0.032		0.027	
	p	>0.7		<0.01		<0.01		<0.02		>0.3		>0.05	
…Hg	n	12	6	12	6	12	8	12	6	11	5	11	8
	$\bar{x}$	37.3	38.4	43.5	56.2	36.0	46.7	34.7	42.6	35.1	42.7	34.0	34.9
	range	30.1–46.1	30.5–44.5	35.2–55.0	51.5–62.0	28.9–49.0	37.1–9.0	30.3–41.9	38.2–51.0	32.1–45.0	35.9–56.2	30.6–38.4	26.6–39.5
	SD	4.9	4.9	5.4	4.0	5.5	6.8	3.8	4.6	3.8	8.2	2.7	4.3
	$\bar{x}_C - \bar{x}_P$	−1.1		−11.4		−10.7		−7.9		−7.6		−0.9	
	p	>0.6		<0.001		<0.01		<0.01		<0.05		>0.5	

hypoventilation with respiratory acidosis but without interference with intrapulmonary oxygen transfer there seems to be a metabolic effect on the mother judging from umbilical venous blood composition. The standard bicarbonate seems to be higher after the administration of pethidine which may be due to decreased lactic acid production in the mother. This idea is supported by the finding of slightly lower lactate levels in umbilical venous blood after pethidine premedication which might be due either to the sedative effect of the drug on the mother or to a regulating effect on uterine action (*Lindgren* 1966). The stress of delivery with hyperventilation as well as increased uterine activity is known to increase lactic acid production (*Brown Jr* 1966 *Derom* 1964). Actually there seems to be a damping effect on maternal ventilation judging from the P_{CO_2} values in umbilical venous blood.

While the slight foetal depression induced by pethidine does not seem to bear any serious risk as long as the delivery proceeds normally the potential danger lies in the possibility of other asphyxial complications being superimposed, thus changing a mildly depressive agent into a toxic one (*James* 1960 *Kliger* and *Nelson* 1965). This is clearly suggested by the observation that there is on average a two-to-threefold increase in the incidence of infant depression, estimated by Apgar scoring and by the need for resuscitation, in a large series where pethidine was used (*Wearing* and *Love* 1964). These findings agree with other clinical observations that the incidence of infant depression is between 7 and 16 per cent after pethidine medication (*Gilbert* and *Dixon* 1943 *Davis* and *Tupper* 1949 *Winters Garcia* and *Lubin* 1951 *Paterson* and *Prescott* 1954).

It seems reasonable to conclude that even an analgesic generally considered harmless, such as pethidine should not be used in the delivery room without valid indications and without facilities for prompt attention to the newborn infant including specific morphine antagonists (*i.e.* Nalorphine®).

SUMMARY

Postnatal respiratory adjustment as evaluated by blood gases and acid base balance was studied during the first 24 hours of

III *Comparison Between Control (C) and Pethidine (P) Group with Respect to Lactic Acid Concentration*

		Umbilical Vein		10 Min.		30 Min.		60 Min.
		C	P	C	P	C	P	C
e	n	8	4	8	4	8	4	4
l	$\bar{x}$	2.7	2.3	3.6	3.8	2.2	2.1	1 65
	range	3.8–1.5	2.7–2.0	4 7–2.6	4.3–3.1	3 7–1.2	2.3–1.5	2.5–1.2
	SD	0.9	0.3	0.9	0.5	0.7	0.4	0.6
	$\bar{x}_C - \bar{x}_P$	0.3		−0.2		0.1		
	p	>0 4		>0.5		>0.7		

IV *Comparison Between Control (C) and Pethidine (P) Group with Respect to Oxygen Tension and Calculated Anatomical R-L Shunt During Breathing of Pure Oxygen*

		60 Minutes		5 Hours		24 Hours	
		C	P	C	P	C	P
0 % O_2	n	9	6	7	3	8	7
t	$\bar{x}$	323	338	575	532	579	574
	range	540–107	532–110	646–499	544–526	643–488	607 539
	SD	169	166	66	10	48	25
	$\bar{x}_C - \bar{x}_P$	−15		43		5	
	p	>0 8		>0.3		>0.8	
mat	n	9	6	7	3	8	7
t	$\bar{x}$	23.6	22.1	8.5	11 4	8.3	8.2
	range	36 4–10.9	34.9–10 4	14.2–3 4	12.0–10 8	14.5–2 8	11 7 5.2
	SD	9 0	9 6	4 3	0 6	3 6	2.1
	$\bar{x}_C - \bar{x}_P$	1.5		2.9		0 1	
	p	>0 7		>0.3		>0 9	

ing and the calculated R–L shunts the true anatomical R–L shunt is also unaffected by pethidine.

The finding of unchanged oxygenation after pethidine premedication conflicts with reports of decreased oxygen saturation in newborn infants (*Taylor Fumetti Essig*, *Goodman* and *Walker* 1955) but may be explained by differences of method namely the use of indirect ear piece oximetry by the latter authors.

While pethidine thus in the infant induces a relative alveolar

hypoventilation with respiratory acidosis but without interference with intrapulmonary oxygen transfer there seems to be a metabolic effect on the mother judging from umbilical venous blood composition. The standard bicarbonate seems to be higher after the administration of pethidine which may be due to decreased lactic acid production in the mother This idea is supported by the finding of slightly lower lactate levels in umbilical venous blood after pethidine premedication which might be due either to the sedative effect of the drug on the mother or to a regulating effect on uterine action (*Lindgren* 1966) The stress of delivery with hyperventilation as well as increased uterine activity is known to increase lactic acid production (*Brown Jr* 1966 *Derom* 1964) Actually there seems to be a damping effect on maternal ventilation judging from the P_{CO_2} values in umbilical venous blood.

While the slight foetal depression induced by pethidine does not seem to bear any serious risk as long as the delivery proceeds normally the potential danger lies in the possibility of other asphyxial complications being superimposed, thus changing a mildly depressive agent into a toxic one (*James* 1960 *Kliger* and *Nelson* 1965) This is clearly suggested by the observation that there is on average, a two-to-threefold increase in the incidence of infant depression, estimated by Apgar scoring and by the need for resuscitation, in a large series where pethidine was used (*Wearing* and *Love* 1964) These findings agree with other clinical observations that the incidence of infant depression is between 7 and 16 per cent after pethidine medication (*Gilbert* and *Dixon* 1943 *Davis* and *Tupper* 1949 *Winters Garcia* and *Lubin* 1951 *Paterson* and *Prescott* 1954)

It seems reasonable to conclude that even an analgesic generally considered harmless, such as pethidine should not be used in the delivery room without valid indications and without facilities for prompt attention to the newborn infant, including specific morphine antagonists (*i.e* Nalorphine®)

SUMMARY

Postnatal respiratory adjustment, as evaluated by blood gases and acid base balance was studied during the first 24 hours of

life in a group of 8 infants whose mothers had received pethidine as an obstetrical analgesic and in a similar group of 12 infants without premedication.

Pethidine produced a decrease in the effective ventilation as shown by increased CO_2-retention and respiratory acidosis There was however no statistically significant effect either on standdard bicarbonate and lactic acid concentration or on intrapulmonary oxygen transfer The results suggest that the drug's action consists of a selective depression of the respiratory centre of the newborn infant.

REFERENCES

Amias A. G and *Fairbairn D* Brit Med. J *II* 432 1963

Apgar V Anesth. Analg *32* 260 1953

Apgar V Burns J J Brodie B B and *Papper E. M* Amer J Obstet. Gynec. *64* 1368 1952

Brown E. B Jr Ann. NY Acad. Sci. *133* 118 1966

Carmody N C Harp Hosp Bull *21* 17 1953

Crawford J S and *Rudofsky S* Brit. J Anesth. *37* 929 1965

Davis M M and *T pper W R. C* Canad. Med. Ass. J *60* 113 1949

Derom R. Amer J Obstet. Gynec. *89* 241 1964

Gilbert G and *Dixon A.* Amer J Obstet. Gynec. *45* 320 1943

Gordon D W S and *Pinker G D* J Obstet. Gynec. Brit. Comm. *65* 606 1958

James L S Anesthesiology *21* 405 1960

Kliger B and *Nelson H B* Amer J Obstet Gynec. *92* 850 1965

Koch G Pädiat. Pädol. *4* 1968 a

Koch G Resp Physiol. *4* 168 1968 b

Koch G and *Wendel H* Mschr Kinderheilk *115* 82, 1967

Koch G and *Wendel H* Acta Paediat Scand. *56* 10 1967

Koch G and *Wendel H* Biol Neonat. *12* 136 1968

Lindgren L Läkartidningen *63* 3081 1966

Paterson S J and *Prescott F* Lancet, *I* 490 1954

Potts C R. and *Ullery J C* Amer J Obstet. Gynec. *81* 1253 1961

Prod'hom L S Levison H Cherry R. B. Drorbaugh J E. Hubell J P J and *Smith C A.* Pediatrics *33* 682, 1964

Roberts H Kane K. M Percival N and *Please N W* Lancet *I* 128 1957

Roby C and *Schumann W* Amer J Obstet. Gynec. *45* 318 1943

Siggaard-Andersen O Engel K Jørgensen K. and *Astrup P* Scand J Clin. Lab Invest. *12* 172, 1960

Stephen C. R. Nowill W K. and *Martin R. C.*, N Carolina Med. J *13* 616 1952

Taylor E. S. Fumerti H. H. von Estig, L. L. Goodman S. N. and Walker L. C. Amer. J. Obstet. Gynec., 69 348 1955
Ullery J. and Bahr J. R. Amer. J. Obstet. Gynec. 84 1051 1962
Way W. L. Costley E. C. and Way E. L. Clin. Pharmacol. Ther. 6 454 1965
Weering, M. P. and Lowe E. J. Amer. J. Obstet. Gynec., 88 298 1964
Wouters H. C. Garcia C. R. and Lubin S. L. Amer. J. Obstet. Gynec. 61 629 1951

Received on May 10 1967

Johnsson, J E and Stormby N G Acta obst et gynec scandinav 47 35 1968
From the Gynaecologic Department Konung Gustaf V:s Jubileumsklinik (G Gorton M.D) the Cytodiagnostic Department (N G Stormby M.D) and the Institute of Pathology (Prof C. G Ahlström) University of Lund, Sweden

CYTOLOGICAL BRUSH TECHNIQUE IN MALIGNANT DISEASE OF THE ENDOMETRIUM

BY

J E. JOHNSSON AND N G STORMBY

Cytodiagnosis is now regarded as a very reliable method for detecting carcinoma of the uterine cervix Malignant cells from tumours in the uterine cavity may however also be detected in the vaginal smear In most studies cytological examination of vaginal smears revealed carcinoma of the uterine body in 50 to 70 per cent of the cases (*Ayre* 1946 *Scheffey Rakoff* and *Hoffman* 1948 *Papanicolau* 1943 *Burns Hartung* and *Brittingham* 1950 *Fremont Smith* and *Graham* 1950 *Wied* 1953 *Held Schreiner* and *Oehler* 1954 *Boschann* 1958 *Montalvo-Rui* 1958 *Mezzadra* and *Terrano* 1958 *Terrano* 1958 *Turnball* 1958 *Rascoe* 1963 *McGowan* 1964) Some authors have given even better figures varying from 80 to 94 per cent (*Graham* 1958 *von Haam* 1958 *de Laguna* 1958 *Wachtel* 1958) Various modifications have been suggested to improve the diagnostic reliability of the method *Sirtori* and *Morano* (1963) thus massaged the uterus before collecting samples from the vaginal fornix and *Vecchietti* and *Morano* (1958) used induced uterine contraction *i e* vaginal smears were taken about 5 minutes after an intravenous injection of "Methergin This procedure is done under colposcopic control and the fornices are irrigated with physiological saline before the injection A cervical pessary has also been used for collection of secretion and is reported to increase the diagnostic reliability of the method (*Bajardi* 1958)

No figures however have been given in support of this assertion.

Other methods have also been tried, such as aspiration from the endometrium with various instruments. These methods are briefly outlined below Unless otherwise stated the purpose of all of the methods was to obtain suitable material for cytological examination.

1 *Aspiration of secretion from the uterine cavity*

In 1943 *Cary* described a method in which a metal cannula intended for insemination, is introduced into the uterine cavity The secretion is aspirated with a 3 ml syringe Other workers in this field (*Clyman* 1955 *Delleplane* 1958 *Haour* 1958 *Nuovo* 1958 *Terzano* 1958 *Delleplane* and *Russo* 1962) have used similar specially designed metal cannulae sometimes provided with a guard to prevent the instrument from being passed too far into the uterus (*Jordan Bader* and *Nemazie* 1956) In these investigations the diagnostic accuracy was 84 to 96 per cent.

In 1956 *Hecht* reported a series of 901 patients including 52 with carcinoma of the uterine cavity A Killian 2 mm antrum cannula was inserted into the cavity and material was aspirated with a 5–10 ml syringe. The diagnostic accuracy was given as 92.3 per cent (4 false negative and 5 false positive) With vaginal smears alone a correct diagnosis was obtained in only 57 7 per cent.

Wiener Gunn and *Ayre* (1955) stressed that the insertion of metal cannulae as described by Hecht and various endometrial brushes requires some cervical dilatation, and therefore they used a polyethylene catheter instead of a metal cannula.

2 *Sponge swab-biopsy*

Gladstone (1948) wiped the endometrium with a small cellulose or gelatin sponge held by the tips of forceps. The value of the method has been confirmed by other investigators (*Angrist* and *Carpenter* 1950) and in 1957 *Diamond Mitchell* and *Blum* published their experiences with this method in a series consisting of

97 patients The material brought out by the sponge was spread on a slide or the sponge was cut into histological sections All of the samples obtained in this way contained endometrial cells In one case adenocarcinoma was demonstrated and the diagnosis was verified by curettage

3 *Combined aspiration and sponge swab-biopsy*

Bickenbach and *Soost* (1958) described a double cannula. The inner cannula is perforated and coated with a thin sponge. The outer protecting cannula (dimension H 3) is introduced into the internal os after which the internal cannula is passed up into the cavity of the uterine body On aspiration secretion is sucked through the sponge, to which the cells became attached. The purpose of the outer cannula is to avoid contamination by cells from the endocervix

4 *Endometrial lavage*

Morton Moore and *Chang* (1957) described a method in which a thin metal cannula was passed almost to the internal os after which 3–4 ml of physiological sterile saline was injected under low pressure The cannula was then withdrawn and the lavage fluid was aspirated from the posterior fornix The sample was centrifuged and conventional smears were prepared. Endometrial washings obtained in this way from 797 patients revealed cancer of the uterine corpus in 26 in whom the diagnosis was confirmed by curettage There were two false negative and 11 false positive diagnoses A later supplementary report in 1959 gave a somewhat lower degree of reliability

Menolier (1962) reported a firm diagnosis in 90 per cent with the same sampling technique in a series of 22 cases of carcinoma of the uterine corpus She also examined histological sections from embedded endometrial material

5 *Brushes*

Brushes of various types have been used In 1955 *Ayre* described a sampling instrument consisting of a brush of natural bristles

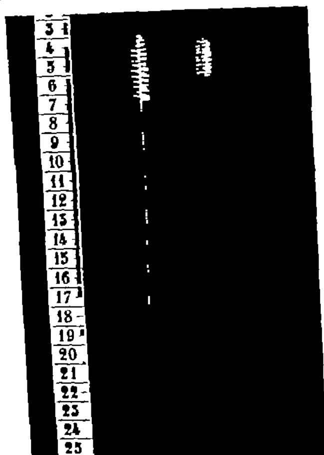


Fig. 1 Two different types of brushes used for endometrial cytology

2 3 cm long enclosed in a protective plastic tube to avoid contamination from the endocervix. The plastic tube was introduced and passed up to a level just above the internal os after which the brush was passed further through the plastic tube into the cavity of the corpus. After rotation of the brush it was then withdrawn through the tube. *Boschann* (1958) used a nylon brush, likewise in a protecting cylinder. The sampling technique was the same as that described by Ayre

Another type of brush (*Fox Turner Johnsson* and *Thornton* 1962) resembling a "bottle brush" with cellulose bristles gave a correct diagnosis in 11 of 12 cases of carcinoma of the uterine corpus On repeat examination the negative sample was also found to contain malignant cells This technique differs from those described above in that the material collected is shaken in physiological saline and then centrifuged Some of the centrifuged deposit is used for the preparation of smears the rest for histological sections

In the present investigation the method of *Fox et al* was evaluated in women with and without known carcinoma of the corpus uteri.

Material and Methods

The method is principally the same as that described by *Fox et al* (1962) The uterine cavity is wiped with a brush, varying in size with the length of the uterus and the cervical canal. The brush (Fig 1) is about 17 cm long and consists of 1 mm twisted brass wire to the end of which are fastened nylon bristles Brushes of two lengths are used *vl* 4 cm and 2 cm The bristles of both models are 8 mm long and 0 12 mm thick.

Sampling is simple and does not require anaesthesia The portio is gripped with a forceps and the cervical canal is carefully probed with an ordinary uterine sound. The brush is then introduced into the cavity of the corpus uteri and turned once about its axis (given a full turn) The brush is then withdrawn and placed in a centrifuge tube (15 ml) with physiological saline. The tube with the brush is sent to the laboratory without delay There the brush is rinsed in a few larger centrifuge tubes (50 ml) likewise containing physiological saline The brushes can be cleaned and autoclaved and reused. The centrifuged deposit is smeared on two slides moistened with albumin glycerin and then fixed and stained in the usual way according to Papanicolaou The sample often contains large fragments which are always embedded in paraffin sectioned (0 4 *i*) stained (haematoxylin and eosin) and examined microscopically

Table I. *Results of Cytological Examinations with the Brush Technique in Known Cases of Endometrial Carcinoma*

Cytol. Grading	No.
I	9
II	9
III	20
IV+V	184
Insufficient material	5
Total	227

The following modification of Papanicolaou-classifications was used.

I. Normal cells (negative)
II. Cells with benign changes (negative)
III. Cells with suspect malignant changes (observation cases curettage indicated as soon as possible as in groups IV and V)
IV Cells strongly suggestive of malignant disease (positive)
V Definitely malignant cells (positive)

Results

1. *Evaluation of the brush technique in a clinical series of known histologically verified cases of malignant endometrial disease*

This part of the material consisted of 227 cases admitted to the Department of radiology because of carcinoma of the uterine body Samples had been collected with the brush 2–6 weeks after primary diagnostic curettage. The results of the cytological examination did not appear to vary with the length of this interval.

The cytological examination revealed no malignant cells (Groups I and II) in 18 cases suspect malignant cells (Group III) in 20 and strongly suspected or definitely malignant cells (Groups IV and V) in 184 cases. In 5 cases the samples were not representative of the endometrium. The results are summarized in Table I

Another type of brush (*Fox Turner Johnsson* and *Thornton* 1962) resembling a bottle brush with cellulose bristles gave a correct diagnosis in 11 of 12 cases of carcinoma of the uterine corpus On repeat examination the negative sample was also found to contain malignant cells This technique differs from those described above in that the material collected is shaken in physiological saline and then centrifuged Some of the centri fuged deposit is used for the preparation of smears the rest for histological sections

In the present investigation the method of *Fox et al* was eval uated in women with and without known carcinoma of the corpus uteri

Material and Methods

The method is principally the same as that described by *Fox et al* (1962) The uterine cavity is wiped with a brush varying in size with the length of the uterus and the cervical canal. The brush (Fig 1) is about 17 cm long and consists of 1 mm twisted brass wire to the end of which are fastened nylon bristles Brushes of two lengths are used *vi* 4 cm and 2 cm The bristles of both models are 8 mm long and 0 12 mm thick

Sampling is simple and does not require anaesthesia. The portio *is gripped with a forceps and the cervical canal* is carefully probed with an ordinary uterine sound. The brush is then in troduced into the cavity of the corpus uteri and turned once about its axis (given a full turn) The brush is then withdrawn *and placed in a centrifuge tube (15 ml) with physiological saline The tube with the brush is sent to the laboratory without delay* There the brush is rinsed in a few larger centrifuge tubes (50 ml) likewise containing physiological saline The brushes can be clean ed and autoclaved and reused *The centrifuged deposit is smeared* on two slides moistened with albumin glycerin, and then fixed and stained in the usual way according to Papanicolaou The sample often contains large fragments which are always em bedded in paraffin sectioned *(0 4) stained (haematoxylin and* eosin) and examined microscopically

Table III. *Comparison Between the Cytological Diagnosis Made on the Basis of Material Collected by the Brush Method and Routine Vaginal Smear*

Cytol Grading	Endometrial Brush	Routine Vaginal Smear
I+II	11	75
III	9	9
IV+V	89	29
Insufficient material	4	
Total	113	113

IV and V) while histological examination of the curettings showed no signs of malignant growth. In 3 cases material obtained from the endometrium by the brush was not representative. The results are summarized in Table II.

In 113 cases samples (routine vaginal smears) were taken for cytological examination from the vagina, portio and cervix immediately before sampling with the brush. While examination of material collected with the brush gave a false negative diagnosis in 11 cases, the corresponding number for smears from the vaginal fornix portio and endocervix was 75. Thus, in only 29 cases (20 per cent) was the ordinary vaginal smear positive. Atypical cells were never found in vaginal smears from women in whom examination of material collected with the brush was negative. The results are summarized in Table III.

II Comparison between results obtained by cytological brush technique and by subsequent diagnostic curettage

This part of the clinical material consisted of 76 patients with bleeding or discharge and five women who had been operated on for ovarian carcinoma. In these 5 samples had been obtained by the brush technique and by curettage before radiotherapy had been started.

In 61 (80 per cent) of the patients the cytological findings were negati e (Groups I and II) In 8 cases (11 per cent) malignant disease was suspected (Group III) and in 7 (9 per

Table II *Comparison Between Cytological and Histological Diagnosis*

Cytol Grading	Histopathological Findings After Curettage				
	No Material	Benign Changes	Atypical	Carcinoma	Total
I+II	1	7		2	10
III		2	3	4	9
IV+V		4	2	55	61
Insufficient material		1		2	3
Total	1	14	5	63	83

The cytological smears showed benign changes in 2 cases and suspected malignant cells in one in all 3 of which histological examination of the embedded material from the brush showed carcinoma On the other hand cytological examination showed malignant cells in one case where the histological examination of the embedded material showed none.

During the latter part of the investigation curettage was done routinely after cytological sampling (83 cases) Curettage was thus done in 10 of the 18 negative cases (Groups I and II) In 8 of these 10 cases histological examination of the curettings showed no signs of residual carcinoma.

Of the 83 cases in which curettage was also done histological examination showed no residual carcinoma in 14 In one case curettage gave no yield. In these cases curettage was carried out 2–6 weeks after the original diagnosis of carcinoma 18 per cent of the cases thus gave no further yield of malignant tumour after the original curettage Since the diagnosis of carcinoma had in each case been made earlier by curettage one might expect that at least some of the cases assigned by cytological examination to Groups I or II were really false negative *i.e* the negative diag nosis was not due to misinterpretation but the previous curettage had removed all cancer growth from the cavity

In 2 cases cytological examination showed a benign picture (Group II) while histopathological examination of the curettings showed carcinoma. In 4 cases the cytological examination gave a diagnosis of carcinoma or strong suspicion of carcinoma (Groups

A 59-year old woman came to the hospital because of menopausal bleeding. Gynaecological examination revealed nothing abnormal and no curettings could be obtained. Sampling with the brush, however produced atypical cells the vaginal smear also showed signs of oestrogenic influence. At follow-up 5 months later bilateral ovarian tumours were palpated and the patient was subjected to hysterectomy with removal of the adnexae. Resampling with the brush again showed atypical cells in the uterus, but histological examination of the surgical specimen showed only normal endometrium. The ovarian tumours proved to be bilateral granulosa cell carcinoma and the atypical cells found in the smears were thus almost certainly derived from these tumours.

In the remaining 5 cases of ovarian carcinoma the endometrium was of normal postclimacteric type.

Of the 7 cases in which cytological examination was positive the diagnosis was verified in 6 by curettage. In the remaining case the endometrium was of normal postclimacteric appearance.

A 53-year old woman was hospitalized because of bloodstained vaginal discharge in the menopause Examination revealed an ovarian tumour judged as inoperable and probably malignant. Samples collected with the brush showed malignant cells of adenocarcinoma type while histological examination of curettings showed only a postclimacteric endometrium. Attempts to obtain material for cytological confirmation by puncture of ovarian tumour proved unsuccessful.

The ovarian tumours were irradiated with a total dose of 2 000 r A follow-up cytological examination with the brush was negative on two occasions but positive again 5 months after the hospital treatment.

In most cases cytological samples were collected simultaneously from the vagina, portio and endocervix. In one of these cases smears of material from the portio showed conclusive evidence of squamous epithelial cancer (Histological examination preinvasive squamous epithelial cancer) The cytological examination of material collected from the endometrium by the brush was negative without contamination with atypical squamous cells.

Table IV *Results of Endometrial Cytology with the Brush as a Primary Diagnostic Tool*

Cytol Grading	No
I	57
II	4
III	8
IV+V	7
Total	76

Table V *Comparison Between Cytological and Histological Diagnosis*

Cytol Grading	Histopathological Findings After Curettage				
	No Material	Benign Changes	Atypical	Carcinoma	Total
I+II	6	55			61
III	2	3	1	2	8
IV+V		1		6	7
Total	8	59	1	8	76

cent) the results of the cytological examination were positive (Groups IV and V) (Table IV)

In those cases assigned cytologically to groups I or II curettage showed no signs of malignant disease in 6 cases no yield was obtained. Cytological examination thus gave no false negative results Of those cases cytologically assigned to group III curettage gave no yield in 2, and in 3 the histopathological diag nosis revealed benign conditions namely endometritis endometrical polyps and postclimacteric endometrium One case showed histologically a slight atypia of the endometrium. In 2 cases histopathological examination revealed adenocarcinoma

In 1 of the 2 cases assigned cytologically to Group III and in which no curettings were obtained the patient had coexisting carcinoma of the vulva and the brush sample may therefore have been contaminated by single atypical cells from this growth A brief history of the second case is given below

Small tissue fragments adhering to the brush and embedded in wax for histological examination have been useful in confirming the diagnosis. However, with increasing experience of the range of variation of the cytological features of the highly differentiated adenocarcinoma histology has less frequently been required. The accuracy of diagnosis naturally increased with experience in the course of the investigation and moderately and poorly differentiated carcinomas never caused any problem.

The investigation showed that the brush technique is suitable for routine clinical work. In our series it caused no complications at all. In most cases sufficient material was obtained from the endometrium. In the latter part of the investigation, when curettage was done routinely immediately after sampling by the brush technique, only 2 out of 83 cases diagnosed cytologically proved to be false negatives. It is remarkable that in 15 out of 83 cases of proven cancer in the first part of the investigation no carcinoma could be demonstrated on repeat curettage. In 4 of these 15 cases cytological examination was positive and could thus be regarded as false positives if the histological findings are taken as evidence of cytodiagnostic reliability. However, since these cases satisfied the same cytological criteria as those in which curettage had verified carcinoma, the cytological diagnoses should not be classified as false positives. This argument suggests that the cytological method is more sensitive than curettage for demonstrating small foci of carcinoma.

In the second part of the investigation adequate material for cytological examination was obtained in all (76) cases and also in the 8 cases where no curettings were obtained. This material however, included those 2 cases where the malignant cells had probably originated from malignant ovarian tumours.

The possibility of contamination by malignant cells from ascitic fluid, ovarian or tubal carcinoma is probably greater in samples collected by the brush technique than by curettage, since isolated malignant cells have a greater chance of being discovered by the former method. There is also an obvious risk of contamination by cells from cancer of the cervix or vagina.

Wide clinical use of the brush technique requires that the sample is protected against autolysis during transport. In the

Discussion

Conventional diagnostic curettage has certain disadvantages It is laborious and requires preparation of the patient with either general or local anaesthesia it requires dilatation of the cervix and the curettings especially when the yield is small, must be collected with care The routine histopathological examination must for practical reasons, be confined to a few paraffin sections from at most a few different depths Moreover especially in the postclimacteric age group the curettings are sometimes too scanty to allow preparation or sections In cases of cancer of the uterine corpus appearing after the climacteric the yield is usually abundant. This implies therefore that the carcinoma is often fairly advanced Early carcinoma of the uterine body by conventional curettage may be missed owing to the loss of small tumour fragments during preparation of the curettings in the clinic and in the pathological laboratory

The cytological brush technique offers advantages over ordinary curettage the brush can be introduced into the uterine cavity without any appreciable discomfort to the patient and without previous dilatation and in the senile uterus as well as in the previously irradiated one the brush method is definitely more gentle the possible risk of spread of tumour cells into the blood and lymph stream is probably smaller than that resulting from curettage

The brush method thus appears to approach the ideal for the diagnosis of malignant tumours in the uterine cavity The cytological diagnosis however is difficult and correct interpretation of the smears requires skill and experience The commonest type of malignant tumour of the endometrium the adenocarcinoma is often highly differentiated with relatively little cytological atypia After the menopause the evaluation of the hormonal activity in the vaginal smear is often of considerable help in the diagnosis of the highly differentiated adenocarcinoma while the same tumour in childbearing age group may be more difficult to diagnose Cases of this type were found mainly in Group III (suspect cancer cells) This however did not delay treatment because even on the slightest suspicion of malignant disease the patient is subjected to prompt conventional diagnostic curettage

Small tissue fragments adhering to the brush and embedded in wax for histological examination have been useful in confirming the diagnosis. However with increasing experience of the range of variation of the cytological features of the highly differentiated adenocarcinoma histology has less frequently been required. The accuracy of diagnosis naturally increased with experience in the course of the investigation and moderately and poorly differentiated carcinomas never caused any problem.

The investigation showed that the brush technique is suitable for routine clinical work. In our series it caused no complications at all. In most cases sufficient material was obtained from the endometrium. In the latter part of the investigation, when curettage was done routinely immediately after sampling by the brush technique only 2 out of 83 cases diagnosed cytologically proved to be false negatives. It is remarkable that in 15 out of 83 cases of proven cancer in the first part of the investigation no carcinoma could be demonstrated on repeat curettage In 4 of these 15 cases cytological examination was positive and could thus be regarded as false positives if the histological findings are taken as evidence of cytodiagnostic reliability However since these cases satisfied the same cytological criteria as those in which curettage had verified carcinoma, the cytological diagnoses should not be classified as false positives This argument suggests that the cytological method is more sensitive than curettage for demonstrating small foci of carcinoma.

In the second part of the investigation adequate material for cytological examination was obtained in all (76) cases and also in the 8 cases where no curettings were obtained. This material however included those 2 cases where the malignant cells had probably originated from malignant ovarian tumours.

The possibility of contamination by malignant cells from ascitic fluid, ovarian or tubal carcinoma is probably greater in samples collected by the brush technique than by curettage since isolated malignant cells have a greater chance of being discovered by the former method. There is also an obvious risk of contamination by cells from cancer of the cervix or vagina.

Wide clinical use of the brush technique requires that the sample is protected against autolysis during transport. In the

present investigation the material for cytological examination was collected at departments in the vicinity of the cytodiagnostic laboratory and it has been shown that samples may be kept for 24 hours in the refrigerator without impairing the possibilities of diagnosis Attempts are being made to find a suitable method of fixation to enable longer transport at room temperature. Fixation in 70 % ethylalcohol is not the answer because the material adheres to the brush in the fixed state and is then difficult to transfer to the slide in adequate amounts.

SUMMARY

On the basis of the present investigation it is concluded that cytological examination of material collected by the brush technique

1 is an excellent supplement to curettage in cases of bleeding and vaginal discharge especially after the menopause
2 can give valuable information in cases where the indication for curettage is doubtful
3 can be used for extensive field examinations in an attempt to diagnose malignant tumours of the uterine corpus early
4 requires more experience and skill on the part of the examiner at the clinic and at the laboratory than the taking and examination of vaginal smears

REFERENCES

Angrist R. J and *Carpenter F* Am. J Obstet Gynecol 59 1029 1950
Ayre J E Am. J Obstet Gynecol 51 743 1946
– Obstet. Gynecol 5 137 1955
Bajardi F Acta Cytolog 2 577 1958
Bickenbach W and *Soost H J* Acta Cytolog 2 575 1958
Boschann H W Acta Cytolog 2 586 1958
Burns E. L Hartung W H and *Bristl gham E.* Arch Patol 50 699 1950
Cary W H Am J Obstet Gynecol 46 422, 1943
Clyman M J Obstet Gynecol 6 258 1955
Delleplane G Acta Cytolog 2 568 1958
Dellep a G and *Russo A.* Minerva Ginec (Torin) 14 325 1962
Diamond B Mitchell N and *Bl m E* Am J Obstet Gynecol 63 C68 1952
Fox C H T rner F G Johnson W L. and *Thornton W N* Am. J Obstet. Gynecol 83 1582 1962

Fremont Smith M and Graham R. M Progress in Gynecol. 1950
Gladstone S A Am. J Med. 5 849 1948
Graham R. M., Acta Cytolog. 2 379 1958
Haour P Acta Cytolog. 2 566 1958
von Haam, E. Acta Cytolog. 2 580 1958
Hecht E. L. Am J Obstet. Gynecol. 71 819 1956
Held, E. Schreiner W E. and Oehler I Schweiz Med. Wochenschr 84 856 1954
Jordan M J Bader G M and Nemazie A. S Obstet. Gynecol. 7 646, 1956
de Laguna J C Acta Cytolog. 2 585 1958
McGowan L Acta Cytolog. 8 434 1954
Menaker N P Am. Med. Women Assoc. 17 735 1962
Mezzadra J M Terzano G Acta Cytolog. 2 582, 1958
Montalvo-Ruiz L. Acta Cytolog. 2 588 1958
Morton D G Moore J G and Chang, N Surg Gynecol. Obstet. 65 113 1957
J Internat. Coll. Surg. 31 137 1959
Nuovo M. V Acta Cytolog 2 570 1958
Papanicolaou G N Am. J Obstet. Gynecol. 46 421 1943
Rascoe R R Am J Obstet. Gynecol. 87 921 1963
Schaffey L Rakoff A. E. and Hoffmann J Am J Obstet. Gynecol 55 453 1948
Sirtori C and Moreno E. Cancer of the uterus. Charles C Thomas Springfield Illinois U.S.A. 1963
Terzano G Acta Cytolog 2 587 1958
Ibidem 2 567 1958
Turnbull L A Ibidem 2 587 1958
Wachtel E Ibidem 2 582, 1958
Vecchietti G and Moreno E Att. di Ost. Gin. quoted by Sirtori & Moreno 4 511 1958
Wied G L Geburtsh u Frauenheilk. 14 422, 1953
Werner M L Gunn S and Ayre J E. Obstet. Gynecol. 5 279 1955

Received on May 10, 1967

present investigation the material for cytological examination was collected at departments in the vicinity of the cytodiagnostic laboratory and it has been shown that samples may be kept for 24 hours in the refrigerator without impairing the possibilities of diagnosis Attempts are being made to find a suitable method of fixation to enable longer transport at room temperature Fixation in 70 % ethylalcohol is not the answer because the material adheres to the brush in the fixed state and is then difficult to transfer to the slide in adequate amounts

SUMMARY

On the basis of the present investigation it is concluded that cytological examination of material collected by the brush technique

1 is an excellent supplement to curettage in cases of bleeding and vaginal discharge especially after the menopause
2 can give valuable information in cases where the indication for curettage is doubtful
3 can be used for extensive field examinations in an attempt to diagnose malignant tumours of the uterine corpus early
4 requires more experience and skill on the part of the examiner at the clinic and at the laboratory than the taking and examination of vaginal smears

REFERENCES

Angrist R J and *Carpenter F* Am. J Obstet Gynecol 59 1029 1950
Ayre J E Am. J Obstet. Gynecol *51* 743 1946
– Obstet Gynecol *5* 137 1955
Bajardi F Acta Cytolog *2* 577 1958
Bi lenbach W and *Soost H J* Acta Cytolog *2* 575 1958
Boschann H W Acta Cytolog *2* 586 1958
Burns E L *Hartung W H* and *Brittingham E* Arch Patol *50* 699 1950
Cary W H Am J Obstet Gynecol *46* 422, 1943
Clyman M J Obstet. Gynecol *6* 258 1955
Delleplane G Acta Cytolog *2* 568 1958
Delleplane G and *Russo A.* Minerv Ginec (Torin) *14* 325 1962
Diamond B *Mitchell N* and *Blum E* Am J Obstet Gynecol *63* C68 1952
Fox C. H *Turner F G* *Johnsson W L* and *Thornton W N* Am J Obstet. Gynecol *83* 1582, 1962

Fremont Smith M. and G *ahan* R. M. Progress in Gynecol. 1950

Gladstone S. A. Am. J. Med. 5 849 1948

Graham R. M. Acta Cytolog. 2 579 1958

Hauer P. Acta Cytolog. 2 566 1958

von Haam E. Acta Cytolog. 2 580 1958

Hecht E. L. Am. J. Obstet. Gynecol. 71 819 1956

Held E. Schreiner W. E. and Oehler I. Schweiz Med. Wochenschr. 84 856 1954

Jordan M. J. Bader G. M. and Nemazie A. S. Obstet. Gynecol. 7 646 1956

de Lagoma J. C. Acta Cytolog. 2 585 1958

McGowan L. Acta Cytolog 8 434 1954

Menoher N. P. Am. Med. Women Assoc. 17 735 1962

Mezzadra J. M. Terzano G. Acta Cytolog. 2 582, 1958

Montalbo-Ruiz L. Acta Cytolog. 2 586 1958

Morton D. G. Moore J. G. and Chang N. Surg. Gynecol. Obstet. 65 113, 1957

J. Internat. Coll. Surg. 31 137 1959

Nuovo M. V. Acta Cytolog. 2 570 1958

Papanicolaou G. N. Am. J. Obstet. Gynecol. 46 421 1943

Rascoe R. R. Am. J. Obstet. Gynecol. 87 921 1963

Scheffey L. Rakoff A. E. and Hoffmann J. Am. J. Obstet. Gynecol. 55 453 1948

Sirtori C. and Moreno E. Cancer of the uterus Charles C. Thomas Springfield, Illinois U.S.A. 1963

Terzano G. Acta Cytolog 2 587 1958

Ibidem 2 567 1958

Turnbull L. A. Ibidem 2 587 1958

Wachtel E. Ibidem 2 582, 1958

Verchuren G. and Moreno E. Att. di Ost. Gin. quoted by Sirtori & Moreno 4 511 1958

Wied G. L. Geburtsh u Frauenheilk 14 422, 1953

Wiener M. L. Gusst S. and Ayre J. E. Obstet. Gynecol. 5 279 1955

Received on May 10 1967

Nieminen, Usko, Acta obst. et gynec. scandinav. 47 52, 1968
From the First Department of Obstetrics and Gynaecology (Professor A. Turunen) University Central Hospital Helsinki Finland

A NEW OPERATION RADIOTHERAPY COMBINATION FOR TREATMENT OF CARCINOMA OF THE CERVIX UTERI

BY

USKO NIEMINEN

Roentgen or other external radiotherapy is administered routinely following extended Wertheim's operation in many clinics throughout the world, including Finland The therapy is usually given by roentgen apparatus from two ventral and two dorsal fields or by the rotation method. High energy therapy (Cobalt unit and Betatron) is also used for after treatment When external radiotherapy is used the treatment intended for the area of the genitalia must be directed through the surrounding tissues This often results in damage to these tissues and prolonged after-effects (dysuria, diarrhoea melaena pain due to intestinal spasm dermatitis etc) The complications are less severe with pendulum roentgen and high energy therapy than with the older roentgen methods The intrapelvic treatment area is often difficult to localise especially in obese patients (*Nieminen* and *Kotsalo* 1966) Even when the area can be pinpointed, a small change in the patient's position may result in the irradiation falling to reach the target area (*Unnérus et al* 1964) The fact that the amount of radiation administered for cancer has been increased in the last few years emphasises the need for greater accuracy to prevent lesions in adjacent tissues

After *Unnérus* and his co-workers (1964 and 1966) had developed an automatic applicator unit for intracavitary radiotherapy in our clinic we thought of using this fairly mobile

apparatus for contact therapy in connection with gynaecologic operations The applicator and its uses during extended Wertheim s operation are described.

Isotope applicator-unit its structure and operation

The device incorporates a spherical lead container (Fig. 1 1) mounted on bearings on a support. When not in use, the isotope (2) is shielded inside the container The path of the isotope consists of a tube (3) bent as shown in the picture there is thus a sufficiently thick layer of lead in every direction to provide a shield against radiation. When irradiation is to be given the isotope is situated with in a sound of stainless steel, curved or straight (4) The container and the sound are connected by a tube (5) which can be bent to the desired shape. This facilitates insertion of the sound into the patient. In addition, the container

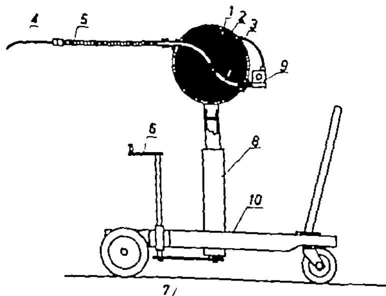


Fig. 1 Sketch of the applicator longitudinal section The preparation on the end of flexible shaft, can be moved by remote control to the tip of the sound.

Nieminen, Usko Acta obst et gynec scandinav 47 92, 1968

From the First Department of Obstetrics and Gynaecology (Professor A. Turunen) University Central Hospital Helsinki Finland

A NEW OPERATION RADIOTHERAPY COMBINATION FOR TREATMENT OF CARCINOMA OF THE CERVIX UTERI

BY

USKO NIEMINEN

Roentgen or other external radiotherapy is administered routinely following extended Wertheim's operation in many clinics throughout the world including Finland. The therapy is usually given by roentgen apparatus from two ventral and two dorsal fields or by the rotation method. High energy therapy (Cobalt unit and Betatron) is also used for after treatment. When external radiotherapy is used the treatment intended for the area of the genitalia must be directed through the surrounding tissues. This often results in damage to these tissues and prolonged after-effects (dysuria diarrhoea, melaena pain due to intestinal spasm dermatitis etc.) The complications are less severe with pendulum roentgen and high energy therapy than with the older roentgen methods. The intrapelvic treatment area is often difficult to localise especially in obese patients (*Nieminen* and *Kotsalo* 1966). Even when the area can be pinpointed, a small change in the patient's position may result in the irradiation failing to reach the target area (*Unnérus et al* 1964). The fact that the amount of radiation administered for cancer has been increased in the last few years, emphasises the need for greater accuracy to prevent lesions in adjacent tissues.

After *Unnérus* and his co-workers (*1964* and *1966*) had developed an automatic applicator unit for intracavitary radiotherapy in our clinic, we thought of using this fairly mobile

Method

Contact therapy is administered in the final stages of extended Wertheim's operation before peritonealisation of the operative area. The sound of the applicator is placed in the area between the stump of the vagina and the lateral wall of the pelvis i.e. in an area to which postoperative radiotherapy is administered from outside. In order to protect the ureters a metal urn which absorbs c. 75 per cent of the radiation is placed between the tip of the sound and the ureter. In addition, the ureter is pushed by a tampon as far from the source of radiation as possible. The intestines are fixed by straps on the cranial side of the operative area, as far from the sound as possible, but avoiding respiratory embarrassment. The patient must be persuaded to relax as completely as possible for the duration of the therapy. The anesthetist looks after the patient with the help of apparatus specially designed for the purpose. The surgical staff, for safety reasons, keeps sufficiently far from the patient during irradiation. The regions of both parametria are treated separately. The treatment time applied by us has ranged during the experimental period from 5 to 10 min., the total treatment time, taking both sides into consideration, being 10–20 min. The amount of radiation during this time to the region of both parametria has been around 500–1000 rad, i.e. the total dose has been 1000–2000 rad.

Discussion

We have sought by the contact therapy described here to find a substitute for a part of external postoperative radiotherapy. Intestines and ureters pushed as far from the source of radiation as possible receive less radiation than if the corresponding dose were administered from outside partly through these organs. Further work will show the proportion of the postoperative radiation dose that can be administered as contact therapy.

Carcinoma of the cervix uteri is known to spread chiefly via the lymph vessels in the lateral parametria, and the majority of recurrences form in the pelvic wall in the region of the os ischii (*Tieve* 1937, *Ries* and *Breitner* 1950, *Belonoschkin* 1955,

plus sound can be rotated round both a vertical and a horizontal axis also to facilitate insertion The height is likewise adjustable by means of a handle (6) which via a chain transmission (7) operates the guide screw inside an upright support (8)

The isotope is moved from the container to the sound and back electrically It is attached to the end of a flexible tube furnished with special toothing The shaft moves when the toothing is engaged by a cog wheel gear (9) driven by an electric motor The extent of the movement is automatically controlled by limit switches placed in the vicinity of the gear It can be started by remote control *e g* from outside the treatment room. The time spent by the isotope in the sound is governed by a pre-set therapy timer When the time is over the isotope returns automatically into the container The location of the isotope is indicated by two signal lights of different colour one for the sound and the other for the container These lamps and the starting device are in a separate light weight control panel

The apparatus as a whole is mounted on a strong carriage with steerable wheels and a brake (10)

Fig 1 is a sketch of the apparatus.

Technical data on the apparatus

Sound diameter 10 mm ∅
Diameter of sphere 320 mm ∅
Sound length 230 mm
Length of the tube (flexible) connecting container and sound 640 mm
Height adjustment range 1290 mm–940 mm
Moving time of the sound 17 sec
Data on the source activity 8 Ci

The mean energy of the cesium unit is 0 662 MeV and the radiation energy consequently lies between that of ^{60}Co and 250 kv roentgen radiation The beta radiation is absorbed in the double steel shield of the source and the walls of the treatment applicators. The radiation source proper consists of a cylinder 11 mm long and 7 mm in diameter

ternal postoperative radiotherapy of the cervix uteri. The advantages of this method are accuracy and a smaller amount of irradiation to the adjacent organs than when the entire course of radiotherapy is administered externally

REFERENCES

Belonoschkin B., Acta radiol. 44 1955

Nieminen U and *Kostiala K.*, Ann. chir et gyn. Fenn. 55 313, 1966

Ries J and *Breitner J* Zschr Geburtsh. u. Gynäk. 133 297 1950

Tiez E., Med. Welt 9 276, 1937

Unnérus C-E Nieminen T Rekonen A and *Vauramo E.*, Ann. chir et gyn. Fenn. 53 480, 1964

Unnérus C-E., *Rekonen A. Vauramo E.*, *Turtola V* and *Kirtalitty K.* Acta obst et gynec scandinav 43 Suppl. 3 1964

Received on Nov 11 1966

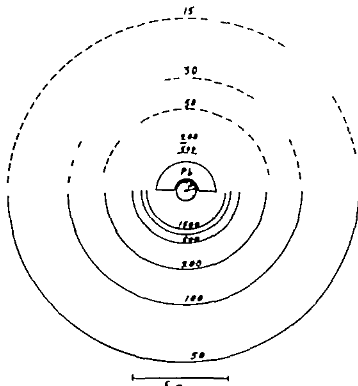


Fig. 2. Isodoses of cesium in the applicator after 10 min. (with metal urn)

Nieminen and *Kotsalo* 1966). Contact therapy makes it possible to direct the radiation precisely to this area.

Our experience of this contact therapy is still limited, but the method appears to be serviceable in theory and it will probably be possible to widen the range of indications. For instance, it is possible that parametrial recurrences of carcinoma of the cervix uteri, the area of the lymph gland metastases encountered at Wertheim's operation, the unexpected discovery of a solid malignant tumour, etc. can be treated during the operation by means of this contact therapy.

As far as I know this method has not been previously used in the treatment of carcinoma colli uteri.

SUMMARY

A method is decribed by which radiotherapy is administered during extended Wertheim's operation, replacing a part of ex

oestrogen compound. However four serious thromboembolic phenomena occurred during the study. Therapy was therefore discontinued in these cases, and the number of cases treated in the second phase with a combination of oestrogen-progestin was not equally divided between the two groups. It was possible to study the liver-function tests in 8 subjects during the treatment with a combination of megestrol acetate-mestranol, in 8 subjects during treatment with ethinyloestradiol-megestrol acetate, and in 6 subjects administered lynoestrenol and mestranol. In each group the steroid first mentioned was administered during the four weeks before the combined treatment. The subjects showed no history or evidence of hepatic biliary diseases or signs of congestive heart failure. During the test period the subjects who were 52 to 78 years of age used no drugs known to produce so-called intrahepatic cholestasis. The average ages in the four initial groups were as follows mestranol group 73 lynoestrenol group 66.3 ethinyloestradiol group 67 and megestrol group 68.6 years.

The following laboratory tests were performed in each group studied serum total bilirubin, thymol turbidity (normal less than 4 MacLagan units) alkaline phosphatase (normal 0.8–2.9 Bessey Lowry units *Bessey et al.* 1946) serum aspartate transaminase (S.G.O.T. normal less than 40 units *Reitman-Frankel* 1957) serum alanine transaminase (S.G.P.T. normal less than 35 units, *Reitman-Frankel* 1957) serum cholesterol prothrombin index and bromsulphthalein retention determined 45 minutes after intravenous injection of B.S.P. at 5 mg/kg body weight. The tests were carried out in the forenoon after overnight fasting. The determinations were done before treatment commenced and repeated at two-weekly intervals, except that the B.S.P. retention tests were performed at intervals of four weeks. If an abnormal result was obtained at the end of the study the test concerned was repeated four weeks later.

Results

After the administration of megestrol acetate an increase in B.S.P. retention from the base line value of 8 per cent to 12.4

Eisalo, Antti, Heino, Arvo and Räsänen Veikko, Acta obst. et gynec. scandinav. *47* 58, 1968
From the Koskela Hospital Helsinki Finland

OESTROGEN PROGESTOGEN AND LIVER FUNCTION TESTS

BY

ANTTI EISALO ARVO HEINO AND VEIKKO RÄSÄNEN

When considering the reason for the abnormal liver-function tests in subjects to whom oral contraceptives have been administered it has been suggested that either the oestrogen or the progestogen component is responsible for the changes in these tests (*Adlercreutz* 1964 *Eisalo et al* 1964 *Boake et al.* 1965 *Stoll et al* 1966) or that a synergetic action of the two components takes place (*Borglin* 1965 *Eisalo et al* 1965)

The present study concerns liver-function tests in subjects who have received synthetic oestrogen progestin and their combinations Synthetic steroids widely used as oral contraceptives were employed in the study

Subjects and Methods

The series consisted of 40 postmenopausal women divided into four groups each comprising 10 subjects. For 28 consecutive days the first group was administered daily 0 15 mg of mestranol (3 *methoxy-17α-ethinyloestradiol*) the *second group* 5 *mg of* lynoestrenol (17α-ethinyloestrenol) the third 0 05 mg of 17α-ethinyloestradiol and the fourth 4 mg of megestrol acetate (17α-acetoxy-6-methylpreg-4 6-dien 3 20-dione) One woman refused to take mestranol on trial After the initial phase the experiment was continued for a consecutive period of 28 days by combining the treatment in the oestrogen groups with the progestin compound and in the progestin groups with the

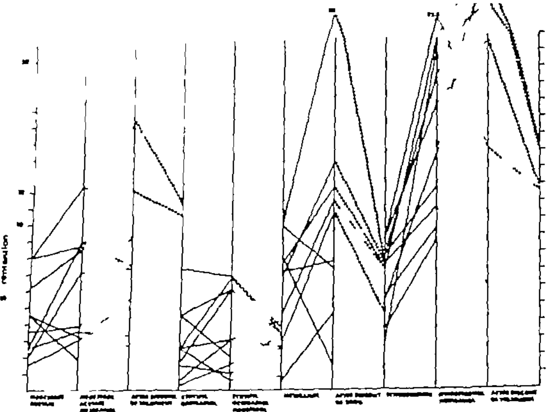


Fig. 1 Results of B.S.P. retention tests. Each column represents a test period of 28 d ys.

per cent was demonstrated in one case only no other changes were found in the liver-function tests in this case (Fig 1) In all the other cases the laboratory tests stayed within normal limits during treatment with megestrol acetate. At the end of treatment with a combination of megestrol acetate and mestranol the increased B S P retention was no different from that following megestrol acetate treatment alone. In another case, however an abnormal B.S P retention was observed at the end of the administration of a megestrol acetate-mestranol combination (16.3 per cent) All the other liver function tests remained *unchanged in this combined treatment.* Two abnormal *B.S P* values were falling when determined four weeks later after the discontinuation of the combined treatment

The liver function tests were unchanged on treatment with ethinyloestradiol and further when treated with a combination of ethinyloestradiol and megestrol acetate (Fig 1)

In the mestranol group the base line values were normal with the exception of two cases with slightly elevated B.S P retention (9 8 per cent and 10 0 per cent) In five out of 9 subjects an increase of the B S P retention was observed in the test (Fig 1) A change in the other liver function tests was encountered in one case only In this case after the period of two weeks of treatment with mestranol the S G O T S G P T and alkaline phosphatase rose from the base line values of 25 units 30 units and 1 7 units to 140 units 315 units and 3 5 units respectively Thereafter the serum transaminase determined on every fourth day declined gradually At the end of treatment the S G O T was 37 units, S G.P T 67 units and alkaline phosphatase 2.2 units despite the rise in the B.S.P retention from the starting value of 10 0 per cent to 23 0 per cent In four subjects the administration of a combination of mestranol lynoestrenol was discontinued after 2 weeks because of thromboembolic occurrences Therefore the liver function tests except that of B.S P retention were performed at this time In three subjects the liver function tests had remained unchanged but in the fourth case who had already reacted to mestranol there were changes in the serum transaminases and in the alkaline phosphatase the S G O T S G P T and alkaline phosphatase rose again after

Table I. *Laboratory Tests in Subjects Treated With Lynoestrenol and With Combination of Lynoestrenol-Mestranol. Values Determined at the End of the Test Period*

		Age	SGOT (Normal < 40 Units)	SGPT (Normal < 35 Units)	Alk. Phosph. (Normal < 2.9 Units)	BSP Retent
1	Basal value	68	32	15	1.7	7.5
	Lynoestrenol		35	37	2.4	10.8
	Lynoestr. + mestranol		65	125	1.8	6.0
	After discontinuance		35	40		
2	Basal value	60	29	20	1.8	8.0
	Lynoestrenol		26	18	1.5	18.8
	Lynoestr. + mestranol		162	214	1.6	26.2
	After discontinuance		23	32		14.0
3	Basal value	63	32	30	2.5	9.0
	Lynoestrenol		20	21	2.5	17.0
	Lynoestr. + mestranol		33	52	2.3	20.6
	After discontinuance			23		11.8
4	Basal value	74	20	14	2.0	7.0
	Lynoestrenol		29	39	3.4	21.0
	Lynoestr. + mestranol		262	475	3.8	45.0
5	Basal value	77	26	13	1.9	9.0
	Lynoestrenol		47	65	2.3	22.5
	Lynoestr. + mestranol		33	29	2.0	14.2
	After discontinuance					11.6
6	Basal value	65	24	11	2.0	5.2
	Lynoestrenol		16	12	1.9	14.0
	Lynoestr. + mestranol		88	128	2.9	27.0
	After discontinuance		20	23	1.9	14.0

lead to disturbances in B.S.P. metabolism (*Mueller* and *Kappas* 1964) but not in doses of 2.5 mg oestradiol daily for ten days in women of childbearing age (*Kleiner et al.* 1965). The difference in the molecular structure of the tested oestrogen components exists only at carbon atom 3, mestranol possessing a methoxy radical instead of a hydroxyl group. This difference may affect B.S.P. metabolism and probably is not dose-dependent (0.15 mg mestranol, 0.05 mg ethinyloestradiol daily in this study).

two weeks from 37 units to 255 units, from 67 units to 380 units and from 2.2 units to 6.5 units Thus in this subject the changes in the liver-function tests following treatment with the mestranol lynoestrenol combination resembled completely those following mestranol treatment alone The abnormal values obtained with mestranol or combined mestranol-lynoestrenol reverted to normal limits when assessed 4 weeks after the cessation of the treatment.

The base line values before lynoestrenol treatment was started were within normal limits with the exception of two cases with a B.S.P retention of 9 0 per cent. The clearest changes in the liver-function tests appeared after treatment with lynoestrenol and were almost exclusively restricted to alterations in B.S.P retention (Fig 1) Eight out of ten cases revealed an increase in B.S P retention but in only two subjects slightly elevated serum transaminases (under 70 units) were found at the end of the lynoestrenol administration, and in one the value of alkaline phosphatase increased from 2.0 units to 3 4 units The greatest changes in the liver-function tests occurred in six subjects receiving a lynoestrenol-mestranol combination immediately after lynoestrenol administration (Table I) In four cases the S G P T rose over 100 units while no clear changes had been seen after the treatment with lynoestrenol During the combined treatment the B.S.P retention remained unchanged in one case declined in one and increased in four as compared with the B.S P values at the end of lynoestrenol treatment The abnormal laboratory test values obtained tended to decline towards the normal levels during a period of four weeks.

Discussion

The present study showed that no changes occurred in the liver function tests in the women receiving ethinyloestradiol for 28 consecutive days. In five out of nine subjects following methylated oestradiol administration a moderately increased reversible B.S.P retention developed in a similar period one of these women also showed a transient elevation of serum transaminases. Oestrogen administration in large doses has been reported to

*le I. Laboratory Tests in Subjects Treated With Lynoestrenol and With
ubination of Lynoestrenol-Mestranol. Values Determined at the End of the
t Period.*

		Age	S.G.O.T. (Normal < 40 Units)	S.G.P.T. (Normal < 35 Units)	Alk. Phosph. (Normal < 2.9 Units)	B.S.P. Retent
	Basal value	68	32	15	1.7	7.5
	Lynoestrenol		35	37	2.4	10.8
	Lynoestr. + mestranol		65	125	1.8	6.0
	After discontinuance		35	40		
	Basal value	60	29	20	1.3	8.0
	Lynoestrenol		26	18	1.5	18.8
	Lynoestr. + mestranol		162	214	1.6	26.2
	After discontinuance		23	32		14.0
	Basal value	63	32	30	2.5	9.0
	Lynoestrenol		20	21	2.5	17.0
	Lynoestr. + mestranol		33	52	2.3	20.6
	After discontinuance			23		11.8
1	Basal value	74	20	14	2.0	7.0
	Lynoestrenol		29	39	3.4	21.0
	Lynoestr. + mestranol		262	475	3.8	45.0
5	Basal value	77	26	13	1.9	9.0
	Lynoestrenol		47	65	2.3	22.5
	Lynoestr. + mestranol		33	29	2.0	14.2
	After discontinuance					11.6
6	Basal value	65	24	11	2.0	5.2
	Lynoestrenol		16	12	1.9	14.0
	Lynoestr. + mestranol		69	128	2.9	27.0
	After discontinuance		20	23	1.9	14.0

lead to disturbances in B.S.P. metabolism (*Mueller* and *Kappas* 1964) but not in doses of 2.5 mg oestradiol daily for ten days in women of childbearing age (*Meiner et al.* 1965). The difference in the molecular structure of the tested oestrogen components exists only at carbon atom 3: mestranol possessing a methoxy radical instead of a hydroxyl group. This difference may affect B.S.P. metabolism and probably is not dose-dependent (0.15 mg mestranol, 0.05 mg ethinyloestradiol daily in this study).

The tested progestogen components have different B.S.P. retaining properties. This may be due to the difference in the molecular structure of megestrol acetate and lynoestrenol. Despite the markedly increased B.S.P. retention effected by lynoestrenol, the other liver-function tests remained within normal limits. The progestins of different molecular structures may have a different biliary excretion, reflected as disturbances only in the B.S.P. retention but not in the other laboratory tests. It has been stated that 6-methyl 17α-acetoxyprogesterone resembling megestrol acetate in molecular structure, is excreted similarly to progesterone (*Peterson* 1965). However conclusions cannot be drawn because of the complete lack of knowledge concerning the metabolic fate of progestins of different molecular structures and their relationship to B.S.P. metabolism.

The synergism of the oestrogen and progestogen components has been suspected of being responsible for the changes in the liver-function tests (*Borglin* 1965). According to the present study, however, if the oestrogen component (ethinyloestradiol) or the progestogen (megestrol acetate) individually did not cause any disturbances in the laboratory tests, the test values remained within normal limits when they were combined. Further, when mestranol, which when used alone brought about an increase in B.S.P. retention, was combined with megestrol acetate abnormal B.S.P. retention developed in two out of eight subjects. On the other hand, when lynoestrenol, which alone caused most of the increases in B.S.P. retention, was combined with mestranol the B.S.P. retention was elevated still further in four out of six subjects, who also showed changes in the other liver-function tests. Thus the changes in the liver-function tests can be attributed to the influence of oestrogen or progestogen separately rather than to their synergistic action.

SUMMARY

Post-menopausal women were treated with megestrol acetate, 17-ethinyloestradiol, lynoestrenol, mestranol and their combinations for 28 consecutive days and their liver-function tests were followed.

The laboratory tests remained within normal limits in all ten subjects on ethinyloestradiol treatment. In one of ten women the B.S.P. retention increased slightly on the administration of megestrol acetate. Further no abnormalities in the liver-function tests occurred on the administration of ethinyloestradiol-megestrol acetate continued immediately after the ethinyl-oestradiol treatment. In five of nine women treated with mestranol there developed an increased bromsulphthalein (B.S.P.) retention, and in one subject in the same group elevated levels of serum transaminases were present. The highest frequency of increased B.S.P. retention without any other changes in the liver function tests occurred in eight out of ten cases in the group treated with lynoestrenol. When the treatment was continued with a combination of lynoestrenol-mestranol the B.S.P. retention increased still further in four out of six women and in this group the serum transaminases were most clearly elevated.

Acknowledgement

This investigation was supported by a grant from the Sigrid Jusélius Foundation.

REFERENCES

Adlercreutz H. Nord. Med. 72 1133 1964
Bessey O. A., Lowry O. H., and *Brock M. J.* J. biol. Chem. *164* 321 1946
Boake W. C., Schade S. G., Morrissey J. F. and *Schaffner F.* Ann. Int. Med. *63* 302, 1965
Borglin N. E. Brit. Med. J. 1 1289 1963
Eisalo A., Järvinen P. A. and *Luukkainen T.* Ibid. 2 426, 1964
Ibid. 1 1416 1965
Kleiner G. J., Kresch L. and *Arias I. M.* New Engl. J. Med. 273 420 1965
Mueller M. N. and *Kappas A.* J. clin. Invest. 43 1905, 1964
Peterson R. E. In the Biliary System, edited by W. Taylor p. 385 Blackwell Scientific Publ. Oxford, 1965
Reitman S. and *Frankel S.* Amer. J. clin. Path. 28 56 1957
Stoll B. A., Andrews J. T. and *Motteram R.*, Brit. Med. J. 1 960 1966

Received on Nov. 11 1966

Zilliacus, H. Acta obstet. et gynec. scandinav. 47 66, 1968

*From Department I of Obstetrics and Gynaecology (Professor A. Turunen)
University Central Hospital Helsinki Finland*

VAGINAL HYSTERECTOMY

BY

H ZILLIACUS

Introduction

Interest in performing vaginal hysterectomy appears to remain unchanged or even to increase in countries where there is long experience of this operation. An increasing number of enthusiastic reports of favourable results of removal of the uterus by this route are nowadays published from clinics where the training of specialists in operative gynaecology includes this procedure as well

The indications for vaginal hysterectomy vary considerably according to different authorities Prolapse of the uterus with relaxation of the vagina *i.e* cases in which the alternatives are suspension of the uterus or part of it or abdominal hysterectomy and vaginal repair forms a large group In another group of cases the indication for vaginal hysterectomy is uterine disease alone without prolapse or vaginal relaxation. In this group certain anatomical conditions such as a mobile but not too large uterus and a comparatively wide vagina, are usually regarded as prerequisites for the operation

With the proper indications and surgical experience, vaginal hysterectomy offers the patient with prolapse of the uterus considerable advantages, such as less operative trauma and an easier postoperative course as compared with abdominal hysterectomy which in the majority of cases has to be combined with vaginal repair

The indications should not, as Gray (1963) stressed in his monograph, be broadened to include all cases in which hysterectomy has to be performed. Proper selection of the cases to be

submitted to vaginal hysterectomy will give the best results.

In 1963, Gray of U.S.A., reported that the commonest indication, present in 84 per cent of all vaginal hysterectomies performed, was 1st or 2nd degree prolapse of the uterus in combination with vaginal relaxation. In only 2 per cent was uterine disease alone the deciding factor in the choice of this operation. The high frequency of operation in the first group and the low incidence in the second reflect, in a way the technical difficulties encountered when performing the operation under different anatomical conditions.

Of 1438 vaginal hysterectomies reported in 1964 by Centaro DeLaurentiis and Morresi of Italy prolapse of the uterus was the indication in 39.6 per cent, carcinoma of the corpus uteri in 12.5 per cent and other uterine disease in 22.5 per cent. Korpijaakko (1963) of Finland found that in 84.9 per cent of 412 cases of vaginal hysterectomy the indication for the operation was various degrees of descent of the uterus with vaginal relaxation. In 1959 Flämrich, of Austria, reported equal numbers of vaginal hysterectomies performed for prolapse of the uterus and for benign uterine disease alone. In 1959 Fekete of Hungary reported that prolapse of the uterus had been the indication for vaginal hysterectomy in only four out of 500 cases. Uterine disease alone such as carcinoma of the cervix, myoma and metropathy formed the major group of indications. In 1958 Hawksworth and Roux, of England, reported on 1000 cases of vaginal hysterectomy In about 5 per cent of the cases uterine disease was the indication at operation. Uterine prolapse was present in about 25 per cent of all cases operated upon In 1965, Navratil, of Austria, reported on 1536 vaginal hysterectomies performed. The main indications in benign uterine disease were uterine bleeding and fibroids. Navratil pointed out that carcinoma in situ and carcinoma of stage 1 A form new indications for vaginal hysterectomy Hawksworth, in 1965, reported only one death among 3763 vaginal hysterectomies performed, and recommended the operation in cases of prolapse with uterine disease and in prolapse in postmenopausal women. In 1965 Stirling advocated vaginal hysterectomy for the cure of prolapse

Zilliacus, H Acta obst et gynec. scandinav 47 66, 1968

From Department I of Obstetrics and Gynaecology (Professor A. Turunen)
University Central Hospital Helsinki Finland

VAGINAL HYSTERECTOMY

BY

H. ZILLIACUS

Introduction

Interest in performing vaginal hysterectomy appears to remain unchanged or even to increase in countries where there is long experience of this operation. An increasing number of enthusiastic reports of favourable results of removal of the uterus by this route are nowadays published from clinics where the training of specialists in operative gynaecology includes this procedure as well.

The indications for vaginal hysterectomy vary considerably according to different authorities Prolapse of the uterus with relaxation of the vagina, *i.e* cases in which the alternatives are suspension of the uterus or part of it or abdominal hysterectomy and vaginal repair forms a large group In another group of cases the indication for vaginal hysterectomy is uterine disease alone without prolapse or vaginal relaxation. In this group certain anatomical conditions such as a mobile but not too large uterus and a comparatively wide vagina, are usually regarded as prerequisites for the operation

With the proper indications and surgical experience vaginal hysterectomy offers the patient with prolapse of the uterus considerable advantages such as less operative trauma and an easier postoperative course as compared with abdominal hysterectomy which in the majority of cases has to be combined with vaginal repair

The indications should not as Gray (1963) stressed in his monograph, be broadened to include all cases in which hysterectomy has to be performed. Proper selection of the cases to be

same period 1768 abdominal hysterectomies were performed. Because of widely different indications at operation the two techniques are not directly comparable regarding operative results. There was only one death, however in the vaginal hysterectomy series, which means a mortality of 0.2 per cent as compared with 9 deaths or a mortality of 0.5 per cent, in the series of abdominal hysterectomies.

The distribution of the series of vaginal and abdominal hysterectomies over the years 1954–1965 is plotted in Table I. Because the clientele of patients admitted to the hospital does not vary much from one year to another it is admissible to assume from these figures that enthusiasm for performing vaginal hysterectomies was notable in the years 1958 to 1962.

The majority of the patients operated upon were in the age-groups 41–50, 51–60 and 61–70 years. There were 16 patients in the age-group 71–80 years but vaginal hysterectomy was not performed on any over 81 years of age (Table II)

In the relation between parity and frequency of vaginal hysterectomies performed the peak of operations 40.2 per cent, falls in the group of I II parae. The III–IV parae form 34.6 per cent of the cases. There were 9 nulliparae operated upon and 2 cases were in the group of XII–XIII parae

The indications for vaginal hysterectomies performed in this series have been divided according to the principles reported in the current literature i e

Total or almost total prolapse of the uterus with vaginal relaxation cystocele rectocele enterocele 39 cases, 8.4 per cent

2 Partial prolapse of the uterus.

   a with vaginal relaxation, cystocele, rectocele enterocele 25 cases

   b with vaginal relaxation, cystocele rectocele, enterocele and/or with combined syndrome (Gray) cervicitis, laceration, retroversion, fibrosis myoma, adenomyosis, metrorrhagia dysmenorrhoea, urinary incontinence and carcinoma
   255 cases

Table I *Vaginal and Abdominal Hysterectomies Performed in the Years 1954–1965 at Department I of Obstetrics and Gynaecology of the University Central Hospital Helsinki*

	Vaginal	Hysterectomy Deaths	Abdominal	Deaths
1954	8		55	2
1955	14		69	2
1956	22	1	116	0
1957	37		74	0
1958	57		96	0
1959	68		197	3
1960	79		181	1
1961	58		168	1
1962	44		146	0
1963	43		189	0
1964	23		256	0
1965	13		221	0
Total	466	1 (0.2 per cent)	1–68	9 (0.5 per cent)

Table II. *Age of the Patients on Whom Vaginal Hysterectomy Was Performed*

Age in Years	Number of Vaginal Hysterectomies
< 20	0
21–30	0
31–40	7
41–50	151
51–60	179
6 –70	
71–80	17
81–90	0

Own investigations

At Department I of Obstetrics and Gynaecology of the University Central Hospitals Helsinki a total of 466 vaginal hysterectomies were performed in the 12 year period 1954–1965 98 of which were operated upon by the present author During the

same period 1768 abdominal hysterectomies were performed. Because of widely different indications at operation the two techniques are not directly comparable regarding operative results. There was only one death however in the vaginal hysterectomy series, which means a mortality of 0.2 per cent as compared with 9 deaths, or a mortality of 0.5 per cent, in the series of abdominal hysterectomies.

The distribution of the series of vaginal and abdominal hysterectomies over the years 1954–1965 is plotted in Table I. Because the clientele of patients admitted to the hospital does not vary much from one year to another it is admissible to assume from these figures that enthusiasm for performing vaginal hysterectomies was notable in the years 1958 to 1962.

The majority of the patients operated upon were in the age-groups 41 50 51–60 and 61–70 years. There were 16 patients in the age-group 71–80 years but vaginal hysterectomy was not performed on any over 81 years of age (Table II)

In the relation between parity and frequency of vaginal hysterectomies performed the peak of operations 40.2 per cent falls in the group of I–II parae. The III–IV parae form 34 6 per cent of the cases. There were 9 nulliparae operated upon and 2 cases were in the group of XII XIII parae

The indications for vaginal hysterectomies performed in this series have been divided according to the principles reported in the current literature *i.e.*

1 Total or almost total prolapse of the uterus with vaginal relaxation cystocele, rectocele enterocele 39 cases, 8.4 per cent
2 Partial prolapse of the uterus.
   a with vaginal relaxation, cystocele rectocele, enterocele 125 cases
   b with vaginal relaxation, cystocele, rectocele enterocele and/or with combined syndrome (Gray) cervicitis, laceration, etroversion fibrosis, myoma, adenomyosis metrorrhagia dysmenorrhoea, urinary incontinence, and carcinoma
   255 cases

Table I *Vaginal and Abdominal Hysterectomies Performed in the Years 1954–1965 at Department I of Obstetrics and Gynaecology of the University Central Hospital Helsinki*

	Vaginal	Hysterectomy Deaths	Abdominal	Deaths
1954	8		55	2
1955	14		69	2
1956	22	1	116	0
1957	37		74	0
1958	57		96	0
1959	68		197	3
1960	79		181	1
1961	58		168	1
1962	44		146	0
1963	43		189	0
1964	23		256	0
1965	13		221	0
Total	466	1 (0.2 per cent)	1768	9 (0.5 per cent)

Table II *Age of the Patients on Whom Vaginal Hysterectomy Was Performed*

Age in Years	Number of Vaginal Hysterectomies
< 20	0
2 –30	0
31–40	7
41–50	151
51–60	79
6 –70	112
71–80	7
81–90	0

Own investigations

At Department I of Obstetrics and Gynaecology of the University Central Hospitals Helsinki a total of 466 vaginal hysterectomies were performed in the 12-year period 1954–1965, 98 of which were operated upon by the present author During the

same period 1768 abdominal hysterectomies were performed. Because of widely different indications at operation the two techniques are not directly comparable regarding operative results. There was only one death, however in the vaginal hysterectomy series, which means a mortality of 0.2 per cent as compared with 9 deaths or a mortality of 0.5 per cent, in the series of abdominal hysterectomies.

The distribution of the series of vaginal and abdominal hysterectomies over the years 1954–1965 is plotted in Table I. Because the clientele of patients admitted to the hospital does not vary much from one year to another it is admissible to assume from these figures that enthusiasm for performing vaginal hysterectomies was notable in the years 1958 to 1962

The majority of the patients operated upon were in the age-groups 41 50 51–60 and 61–70 years. There were 16 patients in the age-group 71–80 years but vaginal hysterectomy was not performed on any over 81 years of age (Table II)

In the relation between parity and frequency of vaginal hysterectomies performed the peak of operations, 40.2 per cent, falls in the group of I–II parae The III–IV parae form 34.6 per cent of the cases. There were 9 nulliparae operated upon and 2 cases were in the group of XII–XIII parae

The indications for vaginal hysterectomies performed in this series have been divided according to the principles reported in the current literature i.e

1 Total or almost total prolapse of the uterus with vaginal relaxation cystocele rectocele enterocele 39 cases, 8.4 per cent
2 Partial prolapse of the uterus.
   a with vaginal relaxation, cystocele rectocele enterocele 125 cases
   b with vaginal relaxation, cystocele rectocele enterocele and/or with combined syndrome (G r a y) cervicitis, laceration, retroversion, fibrosis myoma, adenomyosis, metrorrhagia dysmenorrhoea, urinary incontinence and carcinoma
   255 cases

a+b=380 cases 81 5 per cent

3 uterine disease alone 47 cases 10 1 per cent.

The figures in the three groups of indication are of about the same magnitude as those reported in the current literature In the series reported by Gray 14 per cent of all the total hysterectomies belonged to group 1 84 per cent to group 2 and 2 per cent to group 3

Among the cases in group 2 were two cases of carcinoma *in situ* two cases of carcinoma of the corporis uteri and one case of a first degree carcinoma of the collum uteri. Because of the prolapse of the uterus the vaginal route for removal of the uterus and the adnexa was considered favourable in the case of carcinoma in situ, operated upon in 1964 and of the cases of corporis uteri operated upon in 1963 and 1964 respectively The case of carcinoma of the collum uteri of stage 1 was operated upon in 1961 according to the technique of Shauta and Amreich (Navratil) Radiation therapy preceded the operation and followed it in the cases of invasive carcinoma At check up in January 1967 all the patients with carcinoma were alive

Vaginal removal of the uterus without vaginal repair was performed in 65 cases In 401 cases the hysterectomy was combined with an anterior and/or posterior colporrhaphy On various indications unilateral salpingo-oophorectomy was performed in 8 cases and bilateral in 3 cases.

During operation no case of shock occurred and there was no immediate mortality Neither excessive bleeding nor uncontrollable haemorrhage occurred during operation

Perforation of the bladder occurred twice The lesions which were sutured immediately did not alter the usual postoperative course There were no lesions of the ureters

In no case were the difficulties encountered during the vaginal operation sufficient to cause a laparatomy

During the postoperative course the following observations were made

There was one death in the series This occurred to a 65 year-old patient on the day of operation from a generalized

haemorrhage of polycythaemic type including symptoms of cerebral haemorrhage

The indication for the vaginal hysterectomy and the vaginal repair was third-degree prolapse of the uterus, chronic cervicitis, cervical polyp and relaxation of the vag na. Preoperative blood count showed that the patient suffered from polycythaemia sedimentation rate 0, haemoglobin 80 G %, white count 16,700 platelet count 545,488 Blood coagulation was normal. bleeding time 3 minutes, coagulation time 8 minutes, prothrombin (Quick) 83 % fibrinogen 290 Mg % Blood pressure 160/ 00 mmHg There was no excessive bleeding during the operation, which was performed in light general anaesthesia. Nor was there unusual bleeding after the operation from the operational area. Eight hours postoperatively however generalized bleeding tendency started. Bleeding from the operational field was still not excessive but large subcutaneous haematomas developed rapidly The patient died two hours later obviously from a cerebral haemorrhage.

In 956 B J 8 k a n, L e u ll and N l l o stated that tissue trauma might easily in cases of polycythaemia, activate the fibrinolytic system of the blood and thus cause generalized fibrinolytic haemorrhage. Even if this was what occurred in the case described the antifibrinolytic agent -aminocaproic acid, discovered by O k m t and O k m t in 962 was not yet available for therapeutic use.

In 51 of the 65 cases in which hysterectomy alone was performed and in 314 cases of the 401 in which both hysterectomy and agina1 repair were undertaken, no postoperative complications occurred. This means a complication-free postoperative period in 78 3 per cent of the cases operated upon.

Elevation of the temperature above 38 0 C occurred in 5 cases when hysterectomy was performed alone and in 87 cases when aginal repair was added to the operation.

Hospital stay exceeded 15 days in 53 cases.

Signs of infection or cellulitis in the operational area occurred in 6 cases.

Postoperative haematomas developed in 5 cases. Resuturation was performed once of a posterior colporrhaphy

Thrombosis of deep calf veins occurred in 3 patients.

There was neither case of pulmonary embolism nor of pneu monia

No case of ileus or of subileus occurred and there was scarcely any postoperati e intestinal distention.

In no cases did a laparatomy ha e to be performed during the postoperative course

No sign of lesion of the bladder or of the ureters occurred in the postoperative period

In the nine cases in which stress urinary incontinence was diagnosed before the operation this had subsided by the time the patient left the hospital

At *follow up examinations* 1 to 2 years after the operation 88 of the patients operated on in the years 1960–1965 because of prolapse of the uterus vaginal relaxation and uterine disease (indication group 2 b) were seen. Of these, eighty-one patients had no complaint to make and at examination no cystocele, rectocele enterocele or prolapse of the vaginal vault could be detected. In three cases there was a prolapse of the vaginal vault with cystocele rectocele and enterocele In two cases there were recurrent enterocele and rectocele and in two cases a second degree stress urinary incontinence had developed.

Discussion

An over-all incidence of 21 7 per cent morbidity after vaginal hysterectomy in the series described is of the same magnitude as reported by many authors The only complication in the vast majority of cases, however was a temperature higher than 38 C In only fourteen cases were the complications more notable cellulitis in 6 cases haematomas in 5 cases and thrombosis in 3 cases. The general haemorrhagic diathesis that developed postoperatively in a case of coexisting polycythaemia and obviously caused the only death in this series cannot be regarded as a complication of this type of operation.

The very favourable results of vaginal hysterectomy both at operation and postoperatively and the strikingly mild post operative course for the patient experienced in this series encouraged us to use this operation when certain indications were present

The main indication for performing a vaginal hysterectomy is naturally the need to remove the uterus. In the 165 cases comprising this series of total subtotal or partial prolapse of the uterus with relaxation of the vagina the aim of removing the uterus was to obtain a better result of the operation for the

prolapse than would have been the case had an operational technique not comprising removal of the uterus been applied. The value of vaginal hysterectomy when performing a vaginal repair for prolapse has been the subject of much discussion in the current literature. Opinion seems to be evenly divided regarding the best operative technique for prolapse: the Manchester operation and vaginal hysterectomy combined with vaginal repair. However, recurrence of the relaxation of the vagina and prolapse of the vaginal vault after vaginal hysterectomy are reported bo be rare when the proper operational technique is applied.

At a follow-up study in 1963, Korpijaakko found symptoms of prolapse in 9.8 per cent of the patients on whom a vaginal hysterectomy had been performed during the years 1943–1959. Hawksworth and Roux (1958) at follow-up examination one year or more after the operation found enterocele in 8.5 per cent of the cases. Symmonds and Pratt (1960) reported vaginal prolapse after vaginal hysterectomy when infection or haematoma complicated the postoperative picture.

In cases of prolapse complicated by additional uterine disease or displacement, vaginal removal of the uterus seemed even more justified, as in 255 cases in this series.

In the 47 cases in which uterine disease alone was the indication for removal of the uterus, the vaginal route was considered the most favourable for the patient.

Because of the ease with which it is possible to remove a sufficient vaginal cuff in connection with a vaginal hysterectomy this operation, as advocated, by Thomson and Lyon (1966) and by Fekete (1956) among others, is obviously the method of choice in many instances of carcinoma in situ of the corpus and collum uteri.

The relative merits of performing vaginal hysterectomy with the Schauta Amreich technique in cases of invasive carcinoma of the uterus must obviously be the subject of renewed discussion since the favourable results reported for pelvic and abdominal lymphadenectomies during the Wertheim operation for uterine carcinoma.

No sign of lesion of the bladder or of the ureters occurred in the postoperative period.

In the nine cases in which stress urinary incontinence was diagnosed before the operation this had subsided by the time the patient left the hospital.

At *follow up examinations* 1 to 2 years after the operation 88 of the patients operated on in the years 1960–1965 because of prolapse of the uterus vaginal relaxation and uterine disease (indication group 2 b) were seen. Of these eighty-one patients had no complaint to make and at examination no cystocele, rectocele, enterocele or prolapse of the vaginal vault could be detected. In three cases there was a prolapse of the vaginal vault with cystocele rectocele and enterocele. In two cases there were recurrent enterocele and rectocele and in two cases a second degree stress urinary incontinence had developed.

Discussion

An over-all incidence of 21.7 per cent morbidity after vaginal hysterectomy in the series described is of the same magnitude as reported by many authors. The only complication in the vast majority of cases however was a temperature higher than 38 C. In only fourteen cases were the complications more notable cellulitis in 6 cases haematomas in 5 cases and thrombosis in 3 cases. The general haemorrhagic diathesis that developed postoperatively in a case of coexisting polycythaemia and obviously caused the only death in this series cannot be regarded as a complication of this type of operation.

The very favourable results of vaginal hysterectomy both at operation and postoperatively and the strikingly mild post operative course for the patient experienced in this series encouraged us to use this operation when certain indications were present.

The main indication for performing a vaginal hysterectomy is naturally the need to remove the uterus. In the 165 cases comprising this series of total subtotal or partial prolapse of the uterus with relaxation of the vagina the aim of removing the uterus was to obtain a better result of the operation for the

prolapse than would have been the case had an operational technique not comprising removal of the uterus been applied. The value of vaginal hysterectomy when performing a vaginal repair for prolapse has been the subject of much discussion in the current literature. Opinion seems to be evenly divided regarding the best operative technique for prolapse the Manchester operation and vaginal hysterectomy combined with vaginal repair However recurrence of the relaxation of the vagina and prolapse of the vaginal vault after vaginal hysterectomy are reported to be rare when the proper operational technique is applied.

At a follow-up study in 1963 Korpijaakko found symptoms of prolapse in 9.8 per cent of the patients on whom a vaginal hysterectomy had been performed during the years 1943–1959. Hawksworth and Roux (1958) at follow-up examination one year or more after the operation found enterocele in 8.5 per cent of the cases. Symmonds and Pratt (1960) reported vaginal prolapse after vaginal hysterectomy when infection or haematoma complicated the postoperative picture.

In cases of prolapse complicated by additional uterine disease or displacement, vaginal removal of the uterus seemed even more justified, as in 255 cases in this series.

In the 47 cases in which uterine disease alone was the indication for removal of the uterus, the vaginal route was considered the most favourable for the patient.

Because of the ease with which it is possible to remove a sufficient vaginal cuff in connection with a vaginal hysterectomy this operation, as advocated, by Thomson and Lyon (1966) and by Fekete (1956) among others, is obviously the method of choice in many instances of carcinoma in situ of the corpus and collum uteri.

The relative merits of performing vaginal hysterectomy with the Schauta-Amreich technique in cases of invasive carcinoma of the uterus must obviously be the subject of renewed discussion since the favourable results reported for pelvic and abdominal lymphadenectomies during the Wertheim operation for uterine carcinoma.

No sign of lesion of the bladder or of the ureters occurred in the postoperative period.

In the nine cases in which stress urinary incontinence was diagnosed before the operation this had subsided by the time the patient left the hospital

At *follow up examinations* 1 to 2 years after the operation 88 of the patients operated on in the years 1960–1965 because of prolapse of the uterus vaginal relaxation and uterine disease (indication group 2 b) were seen. Of these eighty-one patients had no complaint to make and at examination no cystocele rectocele enterocele or prolapse of the vaginal vault could be detected. In three cases there was a prolapse of the vaginal vault with cystocele rectocele and enterocele In two cases there were recurrent enterocele and rectocele and in two cases a second degree stress urinary incontinence had developed.

Discussion

An over-all incidence of 21 7 per cent morbidity after vaginal hysterectomy in the series described is of the same magnitude as reported by many authors The only complication in the vast majority of cases however was a temperature higher than 38 C. In only fourteen cases were the complications more notable cellulitis in 6 cases haematomas in 5 cases and thrombosis in 3 cases The general haemorrhagic diathesis that developed postoperatively in a case of coexisting polycythaemia and obviously caused the only death in this series cannot be regarded as a complication of this type of operation.

The very favourable results of vaginal hysterectomy both at operation and postoperatively and the strikingly mild post operative course for the patient experienced in this series encouraged us to use this operation when certain indications were present.

The main indication for performing a vaginal hysterectomy is naturally the need to remove the uterus In the 165 cases comprising this series of total subtotal or partial prolapse of the uterus with relaxation of the vagina, the aim of removing the uterus was to obtain a better result of the operation for the

tion at follow-up enterocele, prolapse of the vaginal vault and/or urinary incontinence.

In the discussion the favourable results of the operation and the easy postoperative course for the patient are stressed. With the proper indications which chiefly means the need for total removal of the uterus vaginal hysterectomy in the presence of certain anatomical conditions, is, in the opinion of the present author an operation to be recommended.

Operative technique

Preoperative preparation included a survey of general physical condition and correction of possible pathological values concerning fluid balance and blood. Administration of oestrogens to postmenopausal patients for 2–3 weeks prior to operation proved valuable. Preoperative vaginal asepsis included an antibiotic inserted in the vagina overnight before operation.

General anaesthesia given by an anaesthetist was the rule in this series.

The operative procedure described by Heaney (1940) was mostly followed. The cardinal uterosacral and round ligaments and occasionally the ovarian ligaments were sutured to the angles of the vagina. In special cases the round ligaments were attached to the anterior vaginal wall and the uterosacral ligaments to the posterior wall. In some instances the ligaments were drawn under the bladder to support this and sutured together in the midline. Special attention was paid to extra peritonization of the ligaments and vessel pedicles in order to control haemorrhage and secure drainage from the opening left in the retrovaginal space. Oozing from the posterior vaginal vault and cul-de-sac were controlled by interrupted sutures. There was no need to use vasoconstricting agents in this area.

In cases of cystocele, the repair was as a rule performed after closure of the peritoneum. Oozing of blood from this area which might have disturbed the hysterectomy was thus avoided. In suturing the pubocervical fascia special attention was paid to narrowing the region of the internal sphincter in order to prevent or cure urinary stress incontinence.

SUMMARY

In the introduction are mentioned the main indications for performing vaginal hysterectomies as reported by different authors.

A report is given of 466 vaginal hysterectomies performed in the years 1954 to 1965 at Department I of Obstetrics and Gynaecology of the University Central Hospital, Helsinki.

In 65 cases vaginal hysterectomy alone was performed while in 401 cases the hysterectomy was combined with vaginal repair

The indications for the operation were

1 total or subtotal prolapse of the uterus with vaginal relaxation in 39 cases=8 4 per cent.
2 partial prolapse of the uterus
   a. with vaginal relaxation in 125 cases
   b with vaginal relaxation and/or a combined syndrome in 255 cases
   a+b=380 cases=81 5 per cent
3 uterine disease alone in 47 cases=10 1 per cent.

There was one death in the series from generalized haemorrhage in a case of coexisting polycythaemia, *i e* a mortality of 0 2 per cent.

The only complication during operation was perforation of the bladder in two cases

There were no cases of shock or of uncontrollable bleeding during the operation.

Postoperatively there were no complications in 78 3 per cent of the case operated upon, *i.e* a morbidity of 21 7 per cent.

Elevation of the temperature above 38 0 C occurred in 92 cases signs of cellulitis in 6 of haematoma in 5 and of deep thrombosis in the leg in 3 cases. Hospital stay exceeded 15 days in 53 cases.

Examination at follow-up of 88 patients operated upon at least one year earlier revealed 81 patients to be fully cured of the initial prolapse and vaginal relaxation which, in combination with the uterine disease had been the indication for vaginal hysterectomy In 7 cases there were symptoms of vaginal relaxa

Zilliacus, H., Teramo, K., Pöllänen, L., Ottelin, A.-M. and Hakkiluoto, A. Acta obst. et gynec. scandinav. 47 77 1968

From Department I of Obstetrics and Gynaecology (Professor A. Turunen) University Central Hospital Helsinki Finland

CLOTTING AND LYSIS OF BLOOD FROM THE UTERINE CAVITY IN METROPATHY AND ABORTION

BY

H. ZILLIACUS, K. TERAMO, L. PÖLLÄNEN, A.-M. OTTELIN AND A. HAKKILUOTO

As long ago as 1914 Whitehouse showed that menstrual blood withdrawn from the uterine cavity into a glass catheter clotted normally where as menstrual blood collected from the vagina did not clot. These observations were later confirmed by Lozner Taylor and Taylor (1942) among others. The clotting in the uterine cavity is assumed to occur as a consequence of liberation of thromboplastins from the endometrium. The redissolution of the clotted blood is the result of fibrinolysis (Albrechtsen, 956) The fibrinolytic activity of the endometrium is known to depend on a plasminogen activator in the endometrium. Rybo (966) has recently shown that concentration of plasminogen activators in the endometrium increases during the secretory phase, the highest concentration occurring on the first day of the bleeding. In cases of menorrhagia the concentration of plasminogen activators was significantly increased as compared with the values of normal menstrual endometrium. The fibrinolytic activity in the endometrium was higher on the first day of the bleeding than during the premenstrual phase Beller and Graf (1957) considered it possible that the plasmin of menstrual blood directly destroys the fibrinogen before any clotting occurs in the uterine cavity

The aim of this investigation was to study possible clotting and lysis of blood from the uterine cavity in metropathy and abortion.

Posterior colporrhaphy for rectocele or/and enterocele was performed by the usual technique extending the dissection and removal of the mucosa high up in the vagina in order to cure an enterocele or to prevent a later high rectocele or an enterocele. In a number of cases culdeplasty according to McCall (1957) was performed

In addition to routine measures such as early ambulation, postoperative care included the removal of any packings from the vagina within 24 hours, care of a Foley catheter for 4–6 post operative days and administration of a broad-spectrum antibiotic for 5–7 days followed by sulpha medication for 3–4 weeks to prevent late urinary infection.

Acknowledgement

Financial support has been gratefully received from the Sigrid Jusélius Stiftelse

REFERENCES

Björkman S E. Laurell C and *Nilsson I M* Scand J clin. Lab. Invest. 8 304, 1956
Centaro A. Laurentiis G de and *Morresi G* Riv Ostet. Ginec. 19 58 1964
Fekete A. Gynaecologia (Basel) 42 2 1956
Flämrich E., Zbl. Gynäk. 81 537 1959
Gray L. Vaginal Hysterectomy Thomas Springfield, 1963
Hawksworth W J Obstet. Gynaec. Brit Cwlth 72 847 965
Hawksworth W and *Roux J P* J Obstet Gynaec. Brit. Emp. 65 2 4, 1958
Heaney N S Am. J. Surg. 48 88, 1940
Korpijaakko T Ann. Chir Gynaec. Fenn. 52 193 1963
McCall M L Obst. & Gynec. 10 595, 1957
Navratil E The Schauta-Amreich Operation. In Surgical Treatment of Cancer of the Cervix, ed. J V Meigs. Grune and Stratton, New York, 954 p 2 8
Navratil E J Obstet Gynaec Brit Cwlth 72 84 1965
Stirling, H J Obstet. Gynaec. Brit. Cwlth 72 851 1965
Symmonds R E and *Pratt J H* Am. J Obst. & Gynec. 79 899, 1960
Thomson J D and *Lyon J B.* Vaginal Hysterectomy In Management of Genital Prolapse Clin. Obstet. Gynec 9 033, 1966

Received on March 7 1967

Table I *Whole Blood Clotting Time and Whole Blood Lysis Time in Blood Obtained from the Uterine Cavity before and after Curettage of 7 Cases of Metropathic Bleeding*

Case No.	Diagnosis	Clotting Time (Double Determination)				Lysis Time			
		Before Curettage		After Curettage		Before Curettage		After Curettage	
		I	II	I	II	Beginning	Complete	Beginning	Complete
	Hyperpl. endom.	22		20	30	45'	90	5	30
	Endometritis	5	17			5	25	5	5
3	Hyperpl. endom.	26"	33	3	6"	60	80		20
4	Endometritis		6"	22	20		20	5	20
5	Hyperpl. cyst. gland	44	57				5'		5'
6	Endometritis	7	5	43	64	5'	60	5	60
7	Endometritis	47	30				20		

Table II *Whole Blood Clotting Time and Whole Blood Lysis Time in Blood Obtained from the Uterine Cavity before and after Curettage of 6 Cases of Abortion*

Case No.	Diagnosis	Clotting Time (Double Determination)				Lysis Time			
		Before Curettage		After Curettage		Before Curettage		After Curettage	
		I	II	I	II	Beginning	Complete	Beginning	Complete
	Abort. m. III–IV	4	6"			20	20		
	Abort. m. III–IV			3	29				5
3	Abort. mf m. IV	15	20	5		15	5	43	90
4	Recidus post bort.	39		24"	22	5	43	1	5
5	Recidus post abort.	34	35			25'	45'		5
6	Abort. m. II–III		6"	5	24	5	5		5'

Material and methods

Six cases of metropathy and seven cases of abortion were studied with a micro method in this investigation Blood samples were withdrawn from the uterine cavity before exploratory curettage and immediately afterwards. The diagnoses were confirmed by microscopic examination of specimens obtained at curettage

The blood samples were taken with a siliconized glass catheter measuring 33 cm of the standard volume 0 25 ml. To avoid damage to the uterus a polyethylene tube 10 cm in length was connected to the glass catheter and inserted into the uterine cavity A polyethylene tube 50 cm long was connected with the other end of the catheter and the blood was drawn into the catheter

Clotting time was then directly determined (at room temperature) by observing the total clotting of the blood in the catheter The very swift clotting in many instances prevented observations at 37 C.

On observation of the clotting of peripheral blood obtained by venipuncture the mean clotting time was found to be 6' 37 from 30 normal cases. This mean value was obtained by double determinations the extremes being 2 12" and 12 20" The mean value of whole blood clotting time by this method falls within the range of that obtained by the Lee-White method, using ordinary glass tubes. Obviously the small diameter of 0 098 cm of the siliconized glass catheter used here shortens the clotting time With the Lee-White method, using siliconized tubes, the clotting time is 18–25 minutes in normal cases For practical reasons a comparatively long glass catheter has been used in this investigation.

When the catheters were incubated at 37 C it was found that no lysis occurred within 90 minutes. In one case however there was slight lysis after 90 minutes

Prof Cl. v Numers. Pathologist, Obst. & Gyn. Dept., I & II, University Central Hospital.

SUMMARY

Blood samples in siliconized glass catheters were obtained from the uterine cavity before and after curettage in seven cases of metropathy and in six cases of abortion.

It was observed that the whole-blood clotting time was considerably shorter after curettage.

When the same technique for determination of the clotting time was applied to normal peripheral blood, it was found that the blood in all samples from the uterine cavity clotted much faster than normal peripheral blood.

The lysis time of the blood obtained from the uterine cavity especially after curettage in abortion, was considerably shorter than that of normal peripheral blood.

The results are discussed in the light of earlier observation of the clotting mechanism of normal menstrual blood and with regard to the occurrence of thromboplastins and of plasminogen activators in the endometrium.

Acknowledgement

Financial support has been gratefully received from the Sigrid Jusélius Stiftelse

REFERENCES

Albrechtsen O K, Acta endocrinol. 2, 8, 1956
Beller F K and *Graf H* Arch Gynäk 88 4 957
Loomer E L Taylor E and *Taylor F H L*, New England J. Med 226 48 942
Rybo G Acta obst. et gynec. scandinav 45, 429, 1966
Whitehouse B H Lancet 877 914

Received on March, 7 1967

Results

From this investigation it is evident that the clotting of the blood in samples obtained directly from the uterine cavity in cases of metropathy (Table I) is a very rapid process. With the technique employed, the clotting time was between one and 57 seconds The clotting time of normal peripheral blood was 6 minutes and 37 seconds. These observations are in conformity with Whitehouse's classical observation on the intrauterine clotting of menstrual blood.

Of special interest was the finding that the clotting time of the blood obtained from the same cases of metropathy was considerably shorter after curettage. This observation supports the theory that thromboplastic substances are liberated from the endometrium thus initiating intrauterine clotting.

In case no 5 (Table I) the clotting times in double determinations before curettage were 44 and 57 seconds respectively After curettage the clotting time was almost nil, because the lysis which rapidly followed had obviously already started to dissolve the primary coagulum

In all cases there was total lysis of the blood within 5 to 180 minutes in the samples obtained before curettage After curettage the lysis time was considerably shorter varying from some seconds to minutes These observations are in conformity with the findings of plasminogen activators in the endometrium (R y b o 1966)

Analogous observations were made in the cases of abortion observed. The clotting time and lysis time were in general shorter than in the cases of metropathy (Table II)

Case no 3 was exceptional in that the clotting times before curettage were 15 and 20 seconds and after curettage only 5 seconds (one determination) The lysis time however was considerably longer after curettage than before it, although the lysis time in the peripheral blood in this case remained the same Possibly there was direct heavy bleeding from an open vessel into the uterine cavity Because there had been an attempt at criminal abortion followed by infection a slight generalized lysis in the circulating blood was possibly present

SUMMARY

Blood samples in siliconized glass catheters were obtained from the uterine cavity before and after curettage in seven cases of metropathy and in six cases of abortion.

It was observed that the whole-blood clotting time was considerably shorter after curettage.

When the same technique for determination of the clotting time was applied to normal peripheral blood, it was found that the blood in all samples from the uterine cavity clotted much faster than normal peripheral blood.

The lysis time of the blood obtained from the uterine cavity especially after curettage in abortion, was considerably shorter than that of normal peripheral blood.

The results are discussed in the light of earlier observation of the clotting mechanism of normal menstrual blood and with regard to the occurrence of thromboplastins and of plasminogen activators in the endometrium.

Acknowledgement

Financial support has been gratefully received from the Sigrid Jusélius Stiftelse.

REFERENCES

Albrechtsen O. K. Acta endocrinol. 3, 219, 956
Beller F K., and *Graf H.*, Arch. Gynäk *88* 4 1, 957
Lerner *E L.*, *Taylor E* and *Taylor F H L.*, New England J. Med. 226 48 942
Rybo G Acta obst. et gynec scandinav 45 429, 966
Whitehouse B H Lancet 877 914

Received on March, 7 967

Hedberg, E, Rådberg, C and Sannum F Acta obst et gynec scandinav 47 82, 1968
From the Department of Obstetrics and Gynaecology (Professor S Brody) and the Roentgenologic Department (I Wickbom M.D) University of Gothenburg Sweden

RADIOLOGICAL PELVIMETRY IN CASES OF INTRA UTERINE ASPHYXIA

BY

E. HEDBERG C. RÅDBERG AND F SANNUM

The causes of intra uterine asphyxia are manifold and in a given case often multifactorial. In many cases of perinatal death not even a post-mortem examination reveals the true cause of the death It is generally agreed that anoxia is responsible for the great majority of deaths in utero and directly or indirectly for a large part of the mortality and morbidity after birth. Anoxia of the foetus during the second stage of labour as evidenced by change in the foetal heart rate may be the result of direct com pression of the foetal head and brain.

During normal labour the foetal head in cephalic presenta tions is moulded in essentially the same way though the degree of moulding may vary from case to case (*Borell* and *Fernström* 1958) In cases of contracted pelvis the moulding of the foetal head is different from that observed in normal deliveries The degree of elevation of the parietal bones is more pronounced overriding at the sagittal suture is evident and, if the distal part of the pelvis is contracted rupture of the intraoccipital syn chondrosis may occur This abnormal moulding of the foetal head may result in asphyxia of the foetus and death due to intracranial haemorrhage

In order to study to what extent contraction of the distal part of the pelvis may be a cause of foetal intra-uterine asphyxia during the second stage of labour a radiological pelvimetry has been performed on all patients delivered instrumentally because

of foetal asphyxia during the second stage of labour and the results obtained have been compared with those of a randomly selected control series. The study was performed during the years 1963–1965

Material

The material consisted of 105 mothers delivered vaginally by means of forceps or vacuum extractor because of imminent foetal asphyxia during the second stage of labour The control series (98 cases) was randomly selected from mothers with spontaneous deliveries and healthy babies.

The mean age of the mothers in the two series was about the same (26.5 and 26.9 years respectively) Among the mothers of asphyxiated children 97 were primiparae and 8 multiparae. In the control series 27 were primiparae and 70 multiparae. The mean birthweight of the asphyxiated children was 3470 g and, of the controls 3630 g. Of the instrumentally delivered mothers 29 were delivered by means of forceps and 76 by means of vacuum extractor There was one case of perinatal death among the asphyxiated children. In the control series no case of perinatal death occurred.

Method

Early in the puerperium the mothers were examined by means of ortho-diagraphic pelvimetry with special reference to capacity of the distal part of the pelvis

Borell and *Fernström* (1957 1960) have described a method permitting radiological measurement of the distal part of the pelvis. The authors correlated the length of different pelvic diameters with the course of delivery and found a relationship between the capacity of the distal part of the pelvis and the sum of on sagittal and two transverse diameters. This method has later been modified by *Borell* and *Rådberg* (1964) and this ortho-diagraphic modification has been used in the present study By this method the diameters necessary for estimation of the capacity of the pelvis can all be accurately measured with ease on

two films one antero-posterior and one lateral From the antero-posterior view the interspinous and intertuberous diameters are obtained The interspinous diameter is measured between the apices of the ischial spines For calculation of the intertuberous diameter a line is drawn tangentially to the two ischial tuberosities and the points of contact of the line form the limits of the dimension From the lateral view the endpoints of the sagittal diameters of the pelvis and the presenting part of the foetus can be clearly demonstrated For details about the method the reader is referred to the original articles

If the sum of the two transverse and the sagittal diameters of the pelvic outlet exceeds 34 cm disproportion of the distal part of the pelvis is considered most unlikely (*Borell* and *Fernström* 1960) If on the other hand, the sum is less than 32 cm the prognosis for labour must be considered very unfavourable. Measurements between 32 and 34 cm have been defined as border line and the outcome of labour in these cases is uncertain depending mainly on the size of the foetal head. With the modified method used in this investigation the endpoints of the intertuberous diameter are closer to the wall of the birth-canal and therefore the intertuberous diameter is invariably shorter (by about 20 %) Consequently the sum formula of *Borell* and *Fernström* for assessing the capacity of the distal part of the pelvis is reduced the limits for the border line cases being 29.5 to 31.5 cm.

The radiation dose associated with the antero-posterior view using the present technique has been calculated as 0 0002 r to the maternal ovaries and in the case of vertex presentations the foetal gonads receive approximately the same dose The radiation dose with the original method of *Borell* and *Fernström* is about 20 times higher for the same amount of information The lateral view which is common to all radiological pelvimetry examinations gives with our technique a gonadal dose of 0 25–0 35 r

Results

From the data obtained at radiological pelvimetry it is evident that there exists a difference in the capacity of the distal part of

Table I. *Mean Values for Diameters of Pelvic Outlet*

Diameter	Mean Value	
	Instrum. Delivered	Controls
Sag. diameter	11.7	12.3
Intersp. diameter	10.1	10.6
Intertub. diameter	11.3	11.8
Sum of diameters	32.8	34.7

the pelvis between cases delivered instrumentally because of foetal asphyxia, and a control series. This difference is apparent for the mean values of every single diameter of the pelvic outlet and in particular for the sum of the three diameters which difference is statistically significant.

Table I shows the mean values for the single diameters of the pelvic outlet and for the sum of the diameters.

Of special interest is the frequency distribution of the values for the *sum* of pelvic outlet diameters in all cases studied, which is shown in Fig. 1

The figure shows a symmetrical distribution of the values around the mean in both groups, but, in the assisted delivery group the mean is displaced to the left.

In 32 of the 105 patients delivered instrumentally the sum of the three diameters of the pelvic outlet was less than 31.5 cm. In three of these cases the sum was less than 29.5 cm. In the control series a sum of less than 31.5 cm was found in 6 cases out of 98 In one case the sum was less than 29.5 cm.

The mean value for the sagittal diameter of the pelvic inlet was the same in the two groups (12.1 cm)

Discussion

By means of radiology and cephalometry *Borell* and *Fernström* (1958) have shown that normal delivery in vertex presentations always involves moulding of the foetal head. The degree of moulding varies during the passage of the foetal head through the birth canal, being most pronounced at the pelvic inlet when

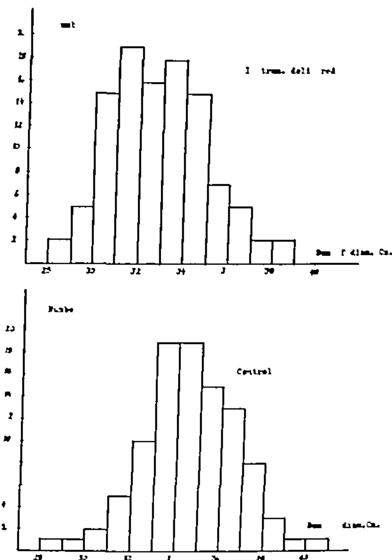


Fig 1 Frequency distribution of the sum of pelvic outlet diameters.

the foetal head is encircled by a zone of increased tone in the uterine wall. In the lower part of the birth canal the degree of moulding diminishes

In cases of pelvic contraction the moulding of the foetal head is different from that in normal deliveries If the distal part of the pelvis is contracted overriding at the sagittal suture and occasionally also of the intra-occipital synchondrosis of the foetal

skull may occur This pathological moulding of the foetal head is often deleterious to the foetus. If the contraction is of minor degree the increased compression of the foetal head may result in changes in foetal heart rate. In cases of advanced pelvic con traction intracranial haemorrhage may damage the respiratory centre directly

It is generally agreed that persistent foetal distress during the second stage of labour as evidenced by significant changes in foetal heart rate is an indication for immediate delivery But it should also be stressed that neither mother nor child should be harmed because of a traumatic delivery

The present investigation has shown that in 105 women, who had instrumental deliveries because of intra-uterine foetal as phyxia, the mean capacity of the distal part of the pelvis was lower than in a randomly selected control series of women who had uncomplicated spontaneous deliveries. The difference was statistically significant. About 30 per cent of the mothers with asphyxiated children had a contracted pelvic outlet shown by subsequent radiological pelvimetry as against 7 per cent of the control series.

It must, of course be realized that changes in foetal heart rate may be caused by a great many factors. It is also impossible to anticipate the outcome of delivery on the basis of pelvic capacity alone The pelvis is but one of many factors involved in the dynamic process of labour Of the mothers of asphyxiated children about 92 per cent were primiparae against 28 per cent in the control series. It is evident that the more rigid soft parts of the pelvis in primiparae also may contribute to the higher fre quency of foetal asphyxia. However if the mean capacity of the distal part of the pelvis is calculated only for the primiparae of the two groups there is still a statistically significant difference between the two groups (32.7 and 34 cm respectively) From the result of the study it may thus be concluded that foetal distress during the second stage of labour may be caused by dis proportion between the foetal head and the distal part of the pelvis.

As a rule there is no time for radiological pelvimetry in cases of acute foetal distress In such cases measurement of the pelvis

ought to be made during the puerperium in order to get an exact knowledge of the pelvic architecture for future deliveries. To get information about the maximal capacity of the distal part of the pelvis the examination should be performed early in the puerperium when the softening of the sacro-iliac joints still persists. In multiparae who have already had difficult forceps or vacuum-extractor deliveries or who have given birth to a stillborn baby *a radiological pelvimetry examination should be performed before* the delivery in order to exclude cephalo-pelvic disproportion

In the present series there was only one perinatal death. However it is not sufficient to measure the successful outcome of delivery only by the birth of a living child. We still know nothing about postnatal growth and development of the children and every traumatic birth involves a risk for future physical or mental retardation

SUMMARY

Ortho-diagraphic pelvimetry with special reference to the capacity of the distal part of the pelvis has been performed in 105 mothers who had assisted vaginal deliveries because of foetal asphyxia during the second stage of labour The findings were compared with those obtained from a randomly selected control series of women who had spontaneous deliveries and healthy children (98 cases)

The mean capacity of the distal part of the pelvis was lower in the women delivered by instruments than in the control series. The difference was statistically significant.

It was concluded that foetal distress during the second stage of labour may be caused by contraction of the distal part of the pelvis

The implications of these findings are discussed

REFERENCES

Borell U and *Fernström I* Acta radiol *47* 365 1957
– Geburtsh. u Frauenh *18* 1156 1958
– Acta radiol *Suppl 191* 1960
Borell U and *Rådberg C* Acta radiol 2 273 1964

Received on April 4 1967

Fjällbrant, Bo. Acta obst. et gynec. scandinav. 47 89, 1968
From the Institute of Medical Microbiology Department of (Professor O Ouchterlony) University of Göteborg, and the of Obstetrics and Gynaecology II (Professor P Bergman) Sahlg[illegible] Institute Göteborg, Sweden

SPERM AGGLUTININS IN STERILE AND FERTILE MEN

BY

BO FJÄLLBRANT

Introduction

That agglutinins against spermatozoa may occur in the human male has been known since 1954 These agglutinins, found in blood serum and seminal plasma and looked upon as auto-antibodies, are as a rule associated with spontaneous agglutination of the spermatozoa in the ejaculate. Despite the work done in this field during the past years the significance of these sperm agglutinins as regards fertility is not yet settled.

Wilson (1954 1956) found three men with sperm agglutinins in their semen and blood among approximately 150 sterility cases and regarded this agglutination of spermatozoa due to antibodies as a newly discovered cause of sterility *Rümke* (1954) found his first two cases with sperm agglutinins in their sera among men with azoo- or extreme oligozoospermia. The extensive investigation of *Rümke* and *Hellinga* (1959) comprised 1913 men in sterile marriages and 416 in fertile marriages. For the determination of the presence and the titre of sperm agglutinins they used the macroscopic, direct agglutination method of Kibrick et al (1952) Normal spermatozoa from several donors were used as antigen for these tests. In the sterile group 0.8 per cent had sperm agglutinins at a titre of 1 16 or lower and 3.2 per cent at a titre of 1 32 or higher In the fertile group they found 4 males with sperm agglutinins, however at low titres (≤1 16) These investigators concluded that sterility might be

the direct result of the action of the autoantibodies in instances where the ejaculates were sufficient in regard to density and motility of spermatozoa. *Phadke* and *Padukone* (1964) however concluded that the presence of sperm agglutinins in the blood serum does not interfere with the fertility of the individual, since they had found 3 fertile men with titres 1 80 1 80 and 1 320 respectively determined by Kibrick s method, among 6 males with sperm agglutinins in a group with obstructive azoospermia relieved by operation. In an investigation of 11 sterile couples of which the male partners had spontaneously agglutinating spermatozoa in their ejaculates and sperm agglutininins in their sera at titres ranging from 1 32 to 1 16384 according to Kibrick s method *Fjällbrant* (1965) proposed that the immunoagglutination of spermatozoa was probably the main cause of sterility in these marriages. Subsequently one of these 11 males with sperm agglutinins proved himself fertile despite titres between 1 128 and 1 1024 and two barren marriages of many years duration (*Fjällbrant* 1967) this finding pointed to the need for further investigation in this field.

Some workers who have used a passive hemagglutination technique instead of methods involving direct agglutination of spermatozoa have found higher incidences of antibodies to spermatozoa and seminal plasma than those stated earlier *Stevens et al* (1965) found that with the passive hemagglutination technique they could measure a natural isoagglutinin which reacted with the ABO blood group substance found in seminal plasma For this reason and because of the discussion during recent years about the role of the blood groups in fertility and sterility ABO blood grouping of the subjects in the present material was performed.

The aim of the present investigation was to determine and compare the incidence of sperm agglutinins in a group of men in sterile marriages with the incidence in a group of men in fertile marriages.

Material and methods

The blood sera of two groups of men one in sterile marriages and the other in fertile marriages were tested for the presence

and titre of agglutinins against spermatozoa from a single donor

The sterile group comprised 400 men, male partners of the couples who attended, because of infertility the Department of Obstetrics and Gynaecology II in Göteborg between March 1965–January 1967 Patients who were sent to the clinic because of suspected immunoagglutination of spermatozoa were not included in order to avoid bias of the random infertile clientele.

The fertile group comprised 500 men, fathers of children born at the department between December 1965–October 1966. All husbands of married women at the puerperal wards during this time were requested to give a blood sample for purposes of research. About one-fourth of them did. From those who proved to have sperm agglutinins another blood sample was taken 4–13 months later The erythrocytes of these men, their wives and children were tested in regard to Rh genotype and the MN Kell and Duffy blood groups see Table I. In no instance did the blood group distribution within the family challenge the paternity of the husband.

Spermatozoa. Fresh ejaculates from the same donor were used throughout the investigation. He belonged to blood group B and was a secretor of B-substance in his seminal plasma. Before use each ejaculate was controlled for volume, sperm density and percentage of motile spermatozoa. The means of these values for 55 ejaculates used in the sperm agglutinin determinations were as follows volume, 5 7 ml (standard deviation 1 45 ml) sperm density 142 million per ml (standard deviation 47 1 million/ml) sperm motility 58 per cent (standard deviation 5.6 per cent) The viscosity was normal the degree of sperm motility was high and spontaneous sperm agglutination was never encountered in the ejaculates used as antigen. The semen was essentially free of leucocytes, erythrocytes, epithelial cells and cellular débris.

The blood samples were centrifuged and the decanted sera kept at − 30 C until examination for agglutinins against spermatozoa

The test for sperm agglutinins was performed with the method described by Kibrick et al. (1952) slightly modified.

The serum was inactivated by heating at 56 C for 30 minutes

the direct result of the action of the autoantibodies in instances where the ejaculates were sufficient in regard to density and motility of spermatozoa. *Phadke* and *Padukone* (1964) however concluded that the presence of sperm agglutinins in the blood serum does not interfere with the fertility of the individual, since they had found 3 fertile men with titres 1 80 1 80 and 1 320 respectively determined by Kibrick s method, among 6 males with sperm agglutinins in a group with obstructive azoospermia relieved by operation. In an investigation of 11 sterile couples of which the male partners had spontaneously agglutinating spermatozoa in their ejaculates and sperm agglutinins in their sera at titres ranging from 1 32 to 1 16384 according to Kibrick s method, *Fjällbrant* (1965) proposed that the immunoagglutination of spermatozoa was probably the main cause of sterility in these marriages Subsequently one of these 11 males with sperm agglutinins proved himself fertile despite titres between 1 128 and 1 1024 and two barren marriages of many years duration (*Fjällbrant* 1967) this finding pointed to the need for further investigation in this field.

Some workers who have used a passive hemagglutination technique instead of methods involving direct agglutination of spermatozoa have found higher incidences of antibodies to spermatozoa and seminal plasma than those stated earlier *Stevens et al* (1965) found that with the passive hemagglutination technique they could measure a natural isoagglutinin which reacted with the ABO blood group substance found in seminal plasma For this reason and because of the discussion during recent years about the role of the blood groups in fertility and sterility ABO blood grouping of the subjects in the present material was performed.

The aim of the present investigation was to determine and compare the incidence of sperm agglutinins in a group of men in sterile marriages with the incidence in a group of men in fertile marriages

Material and methods

The blood sera of two groups of men, one in sterile marriages and the other in fertile marriages were tested for the presence

Table II. *The Distribution of the Sterile and Fertile Groups According to Sperm Agglutinin Titre*

	Neg.	Sperm agglutinin titre (reciprocal)													
		4	8	16	32	64	128	256	512	1024	2048	4096	8192	16384	Total
Sterile	373	3	4	2	1	4	3	1	4	1	1	2	–	1	400
Fertile	487	1	3	3	2		2	–		2		–	–	–	500
Total	860	4	7	5	3	4	5	1	4	3	1	2		1	900

and diluted 1 4 with Baker's solution (buffered glucose) A fresh ejaculate which had just liquified, was diluted with Baker's solution to a sperm density of 40 million per ml. The diluted semen was mixed with an equal volume of 10 per cent gelatin in Baker's solution. Bacto-Gelatin "Difco was used for most of the sera as it was readily liquified and gave a clear solution. An aliquot of 0.3 ml of the semen-gelatin mixture was added to 0.3 ml of the diluted serum. The resultant mixture was transferred to a small precipitation tube (5×65 mm) and incubated at 37 C for 2 hours. The presence of agglutination was assessed macroscopically immediately after the incubation.

For titre determination the sperm agglutinin positive sera, which were kept at −30 C after the screening test, were serially diluted two-fold with Baker's solution. As a rule a series ranging from 1 1 to 1 16384 was prepared. Each dilution was blended with the semen-gelatin mixture and incubated as described above In conformity with Rümke and Hellinga the highest dilution of serum, before the blending with the semen-gelatin mixture that gave an obvious agglutination after the incubation, was registered as the agglutinin titre.

The ABO blood group was determined in all 900 men, in the majority by testing their erythrocytes for antigens, for the rest by testing their serum for agglutinins.

The statistical analysis which involved comparison between distributions was performed with χ^2-tests. A difference was called significant when $p < 0.05$ highly significant when $p < 0.01$ and almost significant when $p < 0.1$

Table I. *The Blood Groups of Husband (♂) Wife (♀) and Child (c) in the 13 Families where the Husband had Sperm Agglutinins*

		ABO	Most probable Rh genotype	MN	Kell	Duffy
I	♂	A	CDe/cde	N	K(−)	Fy(a−)
	♀	A	CDe/cde	M	K(−)	Fy(a−)
	c	A	cde/cde	MN	K(−)	Fy(a−)
II	♂	B	CDe/cde	MN	K(+)	Fy(a+)
	♀	A	CDe/CDe	M	K(−)	Fy(a+)
	c	AB	CDe/cde	M	K(−)	Fy(a+)
III	♂	O	CDe/cde	MN	K(−)	Fy(a−)
	♀	AB	cde/cde	MN	K(−)	Fy(a−)
	c	B	CDe/cde	N	K(−)	Fy(a−)
IV	♂	O	C^wDe/cde	M	K(−)	Fy(a+)
	♀	AB	CDe/cde	M	K(−)	Fy(a+)
	c	B	cde/cde	M	K(−)	Fy(a+)
V	♂	A	CDe/cde	N	K(−)	Fy(a+)
	♀	A	CDe/CDe	M	K(−)	Fy(a−)
	c	A	CDe/CDe	MN	K(−)	Fy(a−)
VI	♂	O	CDe/CDe	MN	K(−)	Fy(a+)
	♀	O	CDe/cde	M	K(−)	Fy(a+)
	c	O	CDe/cde	M	K(−)	Fy(a+)
VII	♂	A	cDe/cde	MN	K(−)	Fy(a+)
	♀	O	CDe/cDE	MN	K(−)	Fy(a+)
	c	A	CDe/cde	MN	K(−)	Fy(a+)
VIII	♂	A	cde/cde	MN	K(−)	Fy(a+)
	♀	AB	CDe/CDe	M	K(−)	Fy(a+)
	c	A	CDe/cde	M	K(−)	Fy(a+)
IX	♂	A	CDe/cde	MN	K(−)	Fy(a−)
	♀	B	CDe/cde	MN	K(+)	Fy(a−)
	c	O	cde/cde	M	K(+)	Fy(a−)
X	♂	O	CDe/cDE	M	K(+)	Fy(a+)
	♀	O	CDe/CDe	M	K(−)	Fy(a−)
	c	O	CDe/CDe	M	K(−)	Fy(a+)
XI	♂	AB	CDe/CDe	MN	K(−)	Fy(a+)
	♀	A	CDe/cDE	N	K(−)	Fy(a−)
	c	A	CDe/CDe	N	K(−)	Fy(a+)
XII	♂	A	cDE/cde	MN	K(−)	Fy(a+)
	♀	B	CDe/cde	MN	K(−)	Fy(a+)
	c	AB	CDe/cDE	N	K(−)	Fy(+)
XIII	♂	O	CDe/CDe	M	K(−)	Fy(a+)
	♀	A	cDE/cDE	MN	K(−)	Fy(a−)
	c	A	CDe/cDE	M	K(−)	Fy(a+)

Table III. The Distribution of Sperm Agglutinin Negative Sera and Sera with Low (≤1 32) and High (≥1 64) Titres in the Sterile and Fertile Groups

Group	Total	Negative sera	Positive sera		
			Low titres	High titres	Total
Sterile	400	373	10	17	27
Fertile	500	487	9	4	13

there were 13 positive sera out of 500 an incidence of 2.6 per cent. The difference between the sterile and fertile groups as regards the incidence of sperm agglutinin positive sera is statistically highly significant ($X^2 = 9.01$ d.f. = 1 $p < 0.01$)

Figure 1 clearly shows the difference as regards the number of positive sera just mentioned, furthermore it is obvious that there was a difference between the sterile and fertile groups in regard to the titre level. This difference was shown to be greatest when a limit was drawn between the titres 1 32 and 1 64 Titres ≤1 32 will in the following text be classified as low and titres ≥1 64 as high. The distribution of negative sera low and high titres is given in Table III

There were 10 low titres in the sterile group and 9 in the fertile group or 2.5 and 1.8 per cent, respectively Statistical analysis showed that this difference between the sterile and fertile groups as regards the incidence of low titres is not significant ($X^2 = 0.53$, d.f. = 1 $p > 0.1$)

Concerning the high titres, there were 17 (4.3 per cent) in the sterile group and only 4 (0.8 per cent) in the fertile group. This difference between the sterile and fertile groups as regards the incidence of high titres is statistically highly significant ($X^2 = 11.61$ d.f. = 1 $p < 0.01$)

When the 13 men in the fertile group who had sperm agglutinins were reinvestigated 4–13 months after the first testing, all of them were still sperm agglutinin positive, see Table IV

The distribution of the ABO blood groups in the sperm agglutinin positive and negative men is given in Table V Statistical analysis showed that there was no significant difference

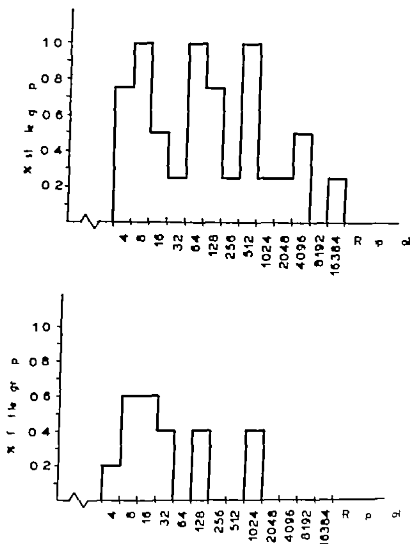


Fig 1 The distribution of sperm agglutinin titres (reciprocals) in the sterile and fertile groups.

Results

The distribution, according to sperm agglutinin titre, of the sera in the sterile and fertile groups is given in Table II The distribution of the sperm agglutinin positive sera is visualized in Figure 1 In the sterile group there were 27 agglutinin positive sera out of 400 an incidence of 6.8 per cent In the fertile group

Table III. The Distribution of Sperm Agglutinin Negative Sera and Sera with Low (≤1:32) and High (≥1:64) Titres in the Sterile and Fertile Groups

Group	Total	Negative sera	Positive sera		
			Low titres	High titres	Total
Sterile	400	373	10	17	27
Fertile	500	487	9	4	13

there were 13 positive sera out of 500, an incidence of 2.6 per cent. The difference between the sterile and fertile groups as regards the incidence of sperm agglutinin positive sera is statistically highly significant ($X^2 = 9.01$, d.f. = 1, $p < 0.01$).

Figure 1 clearly shows the difference as regards the number of positive sera just mentioned; furthermore it is obvious that there was a difference between the sterile and fertile groups in regard to the titre level. This difference was shown to be greatest when a limit was drawn between the titres 1:32 and 1:64. Titres ≤1:32 will in the following text be classified as low and titres ≥1:64 as high. The distribution of negative sera, low and high titres is given in Table III.

There were 10 low titres in the sterile group and 9 in the fertile group or 2.5 and 1.8 per cent, respectively. Statistical analysis showed that this difference between the sterile and fertile groups as regards the incidence of low titres is not significant ($X^2 = 0.53$, d.f. = 1, $p > 0.1$).

Concerning the high titres, there were 17 (4.3 per cent) in the sterile group and only 4 (0.8 per cent) in the fertile group. This difference between the sterile and fertile groups as regards the incidence of high titres is statistically highly significant ($X^2 = 11.61$, d.f. = 1, $p < 0.01$).

When the 13 men in the fertile group who had sperm agglutinins were reinvestigated 4–13 months after the first testing, all of them were still sperm agglutinin positive (see Table IV).

The distribution of the ABO blood groups in the sperm agglutinin positive and negative men is given in Table V. Statistical analysis showed that there was no significant difference

Table IV *The Sperm Agglutinin Titres (reciprocals) of the Fertile Group at Parturition (1st Titre) and 4–13 Months Later (2nd Titre)*

	Case No.												
	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII	XIII
1st titre	16	32	4	8	8	16	128	8	1024	32	16	1024	128
2nd titre	4	8	4	8	8	16	16	4	256	64	16	1024	128

Table V *The Distribution of the ABO Blood Groups in the Sperm Agglutinin Positive and Negative Groups Per Cent Figures in ( )*

	A	O	B	AB	Total
Negative	380 (44.2)	347 (40.3)	87 (10.1)	46 (5.4)	860 (100.0)
Positive	20 (50.0)	15 (37.5)	3 (7.5)	2 (5.0)	40 (100.0)
Total	400 (44.5)	362 (40.2)	90 (10 0)	48 (5.3)	900 (100.0)

between the sperm agglutinin positive and negative groups in regard to the ABO blood group distribution ($X^2 = 0\ 68$ d.f $= 3$ $p > 0\ 1$)

Discussion

The sterile group of the present investigation comprised both patients who attended the clinic primarily and patients who were sent to the clinic by practitioners It is probably represen tive of a Swedish urban sterile clientele

The men of the fertile group gave their blood samples volun tarily The inclusion of questionable paternities was as far as possible avoided by accepting only married men and by blood group testing the sperm agglutinin positive males and their families. Another control of the fertile sperm agglutinin positive group was the reinvestigation after some months

Earlier investigators have stressed the importance of using ejaculates with high sperm density and good sperm motility when

working with Kibrick's gelatin agglutination test. In the present investigation the sperm density of the ejaculates used was high, (mean 142 million per ml) the percentage of motile spermatozoa, however was lower (mean 58 per cent) than in preceding works. The ejaculates were continuously checked and samples with markedly deviating values were discarded. Moreover the accuracy of the method was intended to be increased by using the same semen donor throughout the investigation. The use of one donor also made it possible to consider any possible influence of the ABO blood groups.

In the present investigation, Kibrick's method was also standardized in regard to diluents. Kibrick et al. used isotonic sodium chloride as diluent of the serum and the gelatin, and diluted the semen with this medium or Baker's solution. *Rümke* and *Hellinga* (1959) diluted the serum with saline the semen with Baker's solution and the gelatin with saline or Baker's solution. In the present investigation Baker's solution was chosen as diluent of the gelatin and the semen, because isotonic saline *per se* is toxic to spermatozoa. There was however in the final semen-gelatin-serum mixture a higher pH, particularly at high dilutions and a smaller variation of pH within the series if saline was replaced by Baker's solution as diluent also of the serum. Thus Baker's solution was chosen as diluent of the semen, gelatin and serum.

Inactivation of the sera to be tested is essential, as the agglutinins are accompanied by immobilizing antibodies, the activity of which is greatly enhanced by complement.

There are many reasons why the screening of the sera for sperm agglutinins was done at the dilution 1 4 Kibrick et al. found that nonspecific agglutination sometimes occurred at low serial dilutions, and Rümke and Hellinga sometimes observed a prozone effect with undiluted sera. According to the author's experience accurate reading is often difficult at serum dilutions below 1 4 because of turbidity At a dilution of 1 4 the sera were generally readable, but in some instances with this dilution there was a turbidity which made it necessary to obtain another blood specimen.

As regards the fertile group the incidence of sperm agglutinin positivity at the time of conception is not known, as the testing

was performed nine months later. It can not be excluded that the agglutinin positive men developed their sperm agglutinins after the conception. On the other hand it is evident that the finding of sperm agglutinins at the time of parturition was not accidental since all men were still agglutinin positive 4–13 months later. Moreover it has been reported that a man can be fertile dispite high sperm agglutinin titres for two years before and one year after conception (*Fjällbrant* 1967). Thus it seems not unreasonable to assume that the fertile men with sperm agglutinins were agglutinin positive also at the time of conception.

In an earlier study (*Fjällbrant* 1965) the ejaculates of 263 men in infertile marriages were screened and those who had an apparent spontaneous sperm agglutination were tested for sperm agglutinins in their serum. In that way the incidence of sperm agglutinin positivity was found to be 3.4 per cent. In the present study where all patients were tested for sperm agglutinins in their blood regardless of the behaviour of their spermatozoa, the incidence of agglutinin positivity in the sterile group was just twice as great, 6.8 per cent. This difference is statistically almost significant ($X^2 = 3.42$, d.f. = 1, $p = 0.07$). In the earlier investigation all sperm agglutinin positive sera, with one exception had high titres but in the present investigation there were many with low titres in the sterile group. This higher number of low titres found with the serological screening is statistically significant ($X^2 = 4.37$, d.f. = 1, $p < 0.05$). There is however no statistically significant difference between the numbers of sera with high sperm agglutinin titres found with seminal and serological screening, ($X^2 = 0.64$, d.f. = 1, $p > 0.1$) although the incidences given in per cent differ (3.0 and 4.3 per cent, respectively).

Rumke and Hellinga studied a like material with methods similar to those employed in the present investigation. A comparison of their results with those of the present study shows a statistically significantly greater incidence of sperm agglutinin positivity in the sterile group of the present investigation ($X^2 = 5.31$, d.f. = 1, $p < 0.05$). This difference was more clear-cut at titres $\leq 1:16$ ($X^2 = 6.91$, d.f. = 1, $p < 0.01$) than with titres $\geq 1:32$ ($X^2 = 1.36$, d.f. = 1, $p > 0.1$) and might be ex

plained by a greater sensitivity of the modification of the Kibrick method employed in the present study

The sperm agglutinin positive sera in the fertile group of Rümke and Hellinga all had titres ≤1 16 In the present study there were several sera with higher titres in the fertile group This difference which is statistically significant ($X^2 = 5.03$ d.f. = 1 $p < 0.05$) seems to be the most remarkable difference between the results of the two investigations. At least 2 of the titres in the fertile group of the present material were so high—1 1024 or 6 steps higher than the highest in the fertile group of Rümke and Hellinga—that the difference cannot be explained only by a greater sensitivity of the method.

As there were no titres ≥1 32 in the fertile group of Rümke and Hellinga, these authors considered titres at this level as significant for males of sterile couples. The results of the present investigation are not so unequivocal. The greatest difference however between the sterile and fertile groups was achieved if the limit was drawn between 1 32 and 1 64 i.e. one serial dilution higher

The ABO blood group distribution was about the same in the sperm agglutinin positive and negative groups Most notable is that the number of sperm agglutinin positive males who belonged to the B group i.e. were devoid of anti-B agglutinins, was within the range which is statistically expected if it is assumed that there should be no difference in regard to the incidence of group B between the sperm agglutinin positive and negative groups. As the spermatozoa used as antigen came from a donor belonging to the B group this would not have occurred if the ABO blood group agglutinins were a primary cause of agglutination in the direct sperm agglutination method adopted.

It can be concluded that the ABO blood groups do not influence the results obtained with Kibrick's gelatin agglutination test. The higher incidence of sperm agglutinin positivity and high agglutinin titres in the sterile than in the fertile group permits the assumption that sperm agglutinins may interfere with fertility The validity of this relationship will be tested by investigating the results of a more detailed clinical examination of the males with sperm agglutinins.

SUMMARY

The sera of 400 men in sterile marriages and 500 men in fertile marriages were investigated for sperm agglutinins with Kibrick's gelatin agglutination test. In the sterile group the incidence of positive sera was 6 8 per cent 2.5 per cent with low titres (≤1 32) and 4.3 with high titres (≥1 64) In the fertile group there was an incidence of 2.6 per cent positive sera, 1 8 per cent with low and 0 8 per cent with high titres. Except in regard to low titres these differences between the sterile and the fertile groups were statistically significant.—The distribution of ABO blood groups was almost the same in the sperm agglutinin positive and negative groups, although semen from the same donor was used throughout the investigation.

Acknowledgements

The blood group determinations were done at the Blood Bank (Chief Dr L. Ryttinger) Sahlgrenska sjukhuset in collaboration with Dr Lars A. Nilsson The statistical calculations were checked by Lector E. Carlström the Department of Statistics University of Goteborg Miss Y Steen performed as technical assistant. The English text was revised by Translator C Wadsworth.—The investigation was supported by grants from the Medical Society of Goteborg and the Faculty of Medicine University of Göteborg

REFERENCES

Fjällbrant B. Immunoagglutination of sperm in cases of sterility Acta obstet gynec. scand. *44* 474 1965

– Fertility in a man autoimmunized to sperm J Reprod. Fertil. *14* 143 1967

Kibrick S Belding, D L and *Merrill B* Methods for the detection of antibodies against mammalian spermatozoa. II. A gelatin agglutination test. Fertil. and Steril. *3* 430 1952

Phadke A. M and *Padukone K.* Presence and significance of autoantibodies against spermatozoa in the blood of men with obstructed vas deferens. J Reprod. Fertil. *7* 163 1964

Rümke P The presence of sperm antibodies in the serum of two patients with oligozoospermia. Vox Sang. (Basel) *4* 135 1954

Rümke P. and *Hellinga* G. Autoantibodies against spermatozoa in sterile men. Amer. J. clin. Path. 32 357 1959

Stevens K. M., *Fost* C. A. and *Bellows* A., Circulating antibodies to human seminal plasma in man. J. Reprod. Fertil. 10 137 1965

Wilson L., Sperm agglutinins in human semen and blood. Proc. Soc. exp. Biol. (N.Y.) 85 652, 1954

Sperm agglutination due to autoantibodies. A new cause of sterility. Fertil. and Steril. 7 262, 1956

Received on June 27 1967

Fjällbrant, Bo, Acta obst et gynec scandinav 47 102, 1968

From the Institute of Medical Microbiology Department of Bacteriology (Professor O Ouchterlony) University of Göteborg and the Department of Obstetrics and Gynaecology II (Professor P Bergman) Sahlgrenska sjukhuset Göteborg, Sweden

INTERRELATION BETWEEN HIGH LEVELS OF SPERM ANTIBODIES REDUCED PENETRATION OF CERVICAL MUCUS BY SPERMATOZOA AND STERILITY IN MEN

BY

BO FJÄLLBRANT

Introduction

A possible relationship between sperm antibodies in the blood and fertility was indicated by *Rümke* and *Hellinga* (1959) and *Fjällbrant* (1968) who in investigations of sterile and fertile men for sperm agglutinins found higher frequencies particularly at high titers in the sera from the sterile groups as compared to the fertile groups A possible explanation of this reduction of fertility in association with sperm antibodies has been presented by *Wilson* (1954 1956) During his thorough studies of three men with sperm agglutinins in their semen and blood he observed at postcoital tests unusually few spermatozoa in the cervical mucus The motility of the sperm was sluggish or absent. At cervical mucus invasion tests he noted that the spermatozoa passed through the interface between semen and mucus but then quickly lost their motility Rumke and Hellinga reported a similar finding at the postcoital test in one case with total autoagglutination

Fjällbrant (1965) in a study of 11 men with serologic sperm antibodies found that the spermatozoa of these men had a reduced mucus invading ability both at postcoital tests and *in vitro* In the two men who had the lowest agglutinin titres and

the lowest sperm immobilizing activity of the serum the penetration ability of the spermatozoa was greater than in the other cases, indicating a possible correlation between the sperm antibody level of the serum and the penetration ability of the spermatozoa.

The aim of the present work was to re-examine by means of systematic study of both fertile and infertile men the possible relationship of high levels of sperm antibodies in the serum, reduced penetration ability of spermatozoa from men with sperm antibodies and sterility

Material

The investigation concerned 36 men with sperm antibodies in their blood, of whom 21 lived in sterile marriages and 15 in fertile marriages. The males in the sterile group had attended the clinic with their wives because of infertility So originally had 4 men in the fertile group but as their wives conceived, they were referred to the fertile group with 11 men whose wives were delivered 5–13 months before the investigation. The Rh genotype as well as the ABO MN Kell and Duffy blood groups were determined in these 11 men and their wives and children in no case did the results challenge the paternity of the husband.

The 36 males investigated included 33 men with sperm antibodies whose screening from large groups of sterile and fertile men was reported earlier (*Fjällbrant* 1968) There were also 3 men with sperm agglutinins who had been sent to the department because of suspected immunoagglutination of sperm.

Methods

Demonstration of sperm antibodies in sera

Test for sperm agglutinins. The sperm agglutinin titre of the sera was determined with the macroscopic, direct sperm agglutination test of *Kibrick et al.* (1952) slightly modified as described in detail in an earlier paper (*Fjällbrant* 1968) Sperm from one and the same donor were used for all the agglutinin tests.

Test for sperm immobilizing antibodies. The sperm immobilizing activity of the sera was determined in the following way

The sera to be tested were inactivated at 56 C for 30 min. In a small precipitation tube were mixed 0.2 ml of the inactivated serum 0.1 ml fresh guinea pig serum with controlled complement activity (4 units[1]) and 0.2 ml fresh semen from the same donor as was used for the agglutinin titre determinations. Incubation occurred at room temperature The mixture was repeatedly examined at times depending on the immobilization rate and the assessed sperm agglutinin titre of the serum. After stirring the mixture to break up any agglutinates present, a drop was transferred to a slide and a cover-glass superimposed. The proportion of motile/immotile spermatozoa was estimated under the microscope The time from the beginning of the incubation, when about 70 per cent of the spermatozoa were motile, until the time when only 10 per cent of the spermatozoa were motile, was accepted as an expression of the immobilizing activity of the serum *i e* its concentration of sperm immobilizing antibodies. This value for sera without sperm antibodies was about 60 hours.

Penetration of cervical mucus by spermatozoa

The cervical mucus penetration ability of spermatozoa was investigated with three methods the slide method, the capillary-tube method of Kremer and the postcoital test. The two former tests were done in all cases the postcoital test was performed in the majority of the sterility cases but only in two cases belonging to the fertile group

The investigations with the slide method and the capillary-tube method were done at the same occasion 2–4 hours after ejaculation with the same mucus sample. The postcoital test was done at another occasion sometimes many months earlier In some instances the agglutinin titre and/or the immobilizing activity were different on the two occasions.

To get suitable cervical mucus for the *in vitro* penetration

[1] 1 unit = the complement activity of a serum which gives 100 per cent hemolysis at a serum dilution of 1 8 but <100 per cent hemolysis at a dilution of 1 16.

studies many cervical mucus samples were taken from women at the expected time of ovulation. The penetrability was tested with sperm from the donor used for the sperm antibody tests. Selected for use were 5 readily penetrated voluminous samples of transparent mucus having a high spinnbarkeit and such a low viscosity that the mucus could be easily drawn up into capillary tubes.

With all three methods for determination of the penetration a reduction of motility of the spermatozoa was often observed in the cervical mucus. These different degrees of motility were not taken into consideration at the reading of the tests.

The slide mucus penetration test. A small drop of cervical mucus was placed on a slide and flattened to a diameter of 5–6 mm by a cover-glass the corners of which were supported by small vaseline pillars. Semen was applied at the edge of the cover-glass and was allowed to flow around the cervical mucus. After 3 hours in a moist chamber at room temperature the extent of sperm penetration was read in the following way (cf. Fig. 1)

Degree 0. Invasive spermatozoa found only in the peripheral mucus.

Degree 1. The majority of the spermatozoa which had invaded the mucus observed at the periphery but some noted in the center of the mucus.

Degree 2. Spermatozoa evenly distributed in the mucus.

The capillary-tube mucus penetration test. This was done according to the method described by *Kremer* (1965) with slight modifications.

A column of cervical mucus, about 40 mm long, was drawn into a capillary tube the inner diameter of which was 0.7 mm. The ends of the tube were sealed with modeling clay. The sealed tube was stored at +4 C until used some days or a few weeks later. Then one of the seals was removed and the other partly pushed into the capillary tube so that a mucus drop the size of a pinhead was formed at the other end. This end was immersed into a semen reservoir. After 3 hours in a moist chamber at +37 C the extent of penetration was read in the following way (cf. Fig. 1)

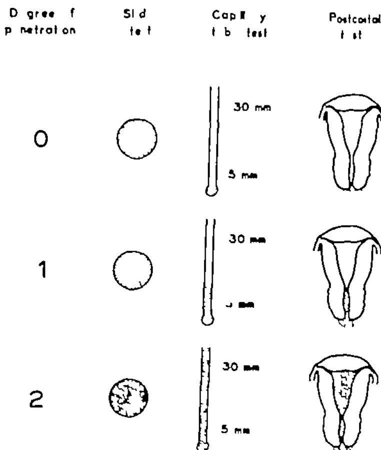


Fig. 1 Diagrammatic representation of the classifications used in the cervical mucus penetration tests.

Degree 0 The foremost spermatozoa were within 5 mm from the immersed end of the tube

Degree 1 The foremost spermatozoa were in the range 6–29 mm from the immersed end.

Degree 2 The foremost spermatozoa were found 30 mm or more from the immersed end.

The postcoital test The postcoital test was performed at the expected time of ovulation within 2–5 hours after coitus Vaginal secretion taken from the posterior fornix cervical secretion taken with forceps first from the lower and then from the upper part of the cervical canal and secretion from the uterine cavity

aspirated with a cannula were examined for the presence of spermatozoa. The extent of the penetration was classified as follows (cf Fig. 1)

Degree 0 Spermatozoa were found, besides in the vaginal secretion, only in the secretion from the lower part of the cervix.

Degree 1 Spermatozoa observed in the secretions from both the lower and the upper part of the cervix.

Degree 2 Spermatozoa were found in the secretions from both the lower and the upper part of the cervix and from the uterine cavity

Statistical calculations

The comparison between distributions was, unless otherwise stated, done with X^2-analysis.

All tests were carried out at a 5 per cent level of significance A difference was called significant when $p < 0.05$ highly significant when $p < 0.01$ and almost significant when $p < 0.1$

Results

Sperm agglutinin titre and fertility

The sera were classified into low (≤ 1 32) and high (≥ 1 64) sperm agglutinin titre categories. It was noted in an earlier paper that these limits provided the greatest difference between the fertile and sterile groups as regards the distribution of high and low titres It can be seen in Table I that the low titres predominated in the fertile group while high titres occurred often in the sterile group This group difference in regard to agglutinin titre in the serum is statistically almost significant ($X^2 = 3.29$ d.f $= 1$ $p = 0.07$)

Sperm immobilizing activity and fertility

The material was divided in regard to immobilizing activity of the sera into one group where the percentage of motile sperm had declined to 10 within 6 hours and another group in which the time was 6 hours or more These groups of sera with high

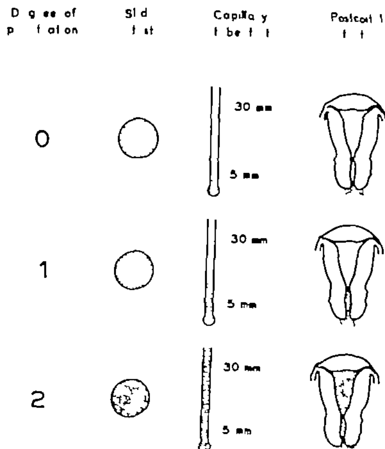


Fig. 1 Diagrammatic representation of the classifications used in the cervical mucus penetration tests.

Degree 0 The foremost spermatozoa were within 5 mm from the immersed end of the tube

Degree 1 The foremost spermatozoa were in the range 6–29 mm from the immersed end.

Degree 2 The foremost spermatozoa were found 30 mm or more from the immersed end.

The postcoital test The postcoital test was performed at the expected time of ovulation within 2–5 hours after coitus Vaginal secretion taken from the posterior fornix, cervical secretion taken with forceps first from the lower and then from the upper part of the cervical canal and secretion from the uterine cavity

aspirated with a cannula were examined for the presence of spermatozoa. The extent of the penetration was classified as follows (cf Fig. 1)

Degree 0 Spermatozoa were found, besides in the vaginal secretion, only in the secretion from the lower part of the cervix.

Degree 1 Spermatozoa observed in the secretions from both the lower and the upper part of the cervix.

Degree 2 Spermatozoa were found in the secretions from both the lower and the upper part of the cervix and from the uterine cavity

Statistical calculations

The comparison between distributions was, unless otherwise stated, done with X^2-analysis.

All tests were carried out at a 5 per cent level of significance. A difference was called significant when $p < 0.05$ highly significant when $p < 0.01$ and almost significant when $p < 0.1$

Results

Sperm agglutinin titre and fertility

The sera were classified into low (≤ 1 32) and high (≥ 1 64) sperm agglutinin titre categories. It was noted in an earlier paper that these limits provided the greatest difference between the fertile and sterile groups as regards the distribution of high and low titres. It can be seen in Table I that the low titres predominated in the fertile group while high titres occurred often in the sterile group. This group difference in regard to agglutinin titre in the serum is statistically almost significant ($X^2 = 3.29$ df $= 1$ $p = 0.07$)

Sperm immobilizing activity and fertility

The material was divided in regard to immobilizing activity of the sera into one group where the percentage of motile sperm had declined to 10 within 6 hours and another group in which the time was 6 hours or more. These groups of sera with high

Table I. *The Distribution of the Material in the Sterile and Fertile Groups According to the Sperm Agglutinin Titres of the Sera*

Group	Agglutinin titre		
	≤1 32	≥1 64	Total
Sterile	9	12	21
Fertile	11	4	15
Total	20	16	36

Table II. *The Distribution of the Material in the Sterile and Fertile Groups According to the Immobilizing Activity of the Sera*

Group	Immobilizing activity		
	≥6 h	<6 h	Total
Sterile	8	13	21
Fertile	12	3	15
Total	20	16	36

and low immobilizing activity are presented in Table II according to fertility

It is clear that the figures were similar to the distribution of the sera in regard to high and low agglutinin titre. There were more sera with high immobilizing activity in the sterile group and more cases with low activity in the fertile group This difference is statistically significant ($X^2 = 6\ 22$, d.f $= 1$ $p < 0\ 05$)

Sperm agglutinin titre and penetration

The degree of the cervical mucus penetration of spermatozoa at different sperm agglutinin titres of the sera is given in Table III In Tables III a and b it is apparent that the penetration degree determined with either the slide or capillary-tube test tended to diminish with rising agglutinin titre In fact there is a statistically significant and negative correlation between the level of the agglutinin titre and the penetration degree $r = -0\ 57$ and $-0\ 55$ respectively

The greatest change in penetration degree occurred between two titre stages 1 32 and 1 64 Cases with penetration degree 0 almost always had titres above and cases with penetration degree 2 below this critical titre stage, while cases with penetration degree 1 were found on both sides. At or below 1 32 the incidence of penetration degree 0 was with the slide method 1 of 20 (95 per cent confidence interval 0 1–24.9 per cent) at or above 1 64 11 of 16 (95 per cent confidence interval 41.3–89 0 per cent) With the capillary-tube method the incidence of penetration degree 0 was 2 of 20 (95 per cent confidence interval 1.2–31 7 per cent) at or below 1 32 but at or above 1 64 it was 10 of 16 (95 per cent confidence interval 35.4–84 8 per cent)

The degree of mucus penetration of spermatozoa, determined with postcoital tests, at different sperm agglutinin titres of the sera is given in Table III c. As mentioned earlier postcoital tests were performed mainly in cases belonging to the sterile group Of 14 sera with high sperm agglutinin titres 11 were related to a penetration degree of 0 Thus the relationship of high agglutinin titre and reduced penetration seems apparent also with this test, although the difference with regard to penetration degree was not statistically significant between the groups with low and high sperm agglutinin titre ($\chi^2 = 1\ 92$, d.f $= 1$ $p > 0\ 1$)

Sperm immobilizing activity and penetration

The degree of cervical mucus penetration of spermatozoa in relation to the immobilizing activity of the serum is given in Table IV It is obvious that the penetration degree tended to diminish with increasing immobilizing activity when it was determined with the slide test, see Table IV a. This tendency was still more pronounced when the penetration degree was determined with the capillary-tube method, see Table IV b There is a statistically significant and negative correlation between the immobilizing activity of the serum and the penetration degree of −0 61 and −0 76, respectively Like the relationship between agglutinin titre and penetration degree there was a sudden change in penetration degree at a certain level of immobilizing activity wi between immobilization of sperm in less

Table III. *The Degree of Cervical Mucus Penetration of the Subject's Spermatozoa in Relation to the Sperm Agglutinin Titre of the Corresponding Serum*

a. Mucus penetration determined with the slide test

Agglutinin titre (Reciprocal)	Penetration degree 0	1	2	Total
1024–2048	3	1		4
256–512	3			3
64–128	5	3	1	9
16–32	1	5	7	13
4–8		6	1	7
Total	12	15	9	36

b. Mucus penetration determined with the capillary tube test

Agglutinin titre (Reciprocal)	Penetration degree 0	1	2	Total
1024–2048	3	1		4
256–512	2	1		3
64–128	5	2	2	9
16–32	1	2	10	13
4–8	1	2	4	7
Total	12	8	16	36

c. Mucus penetration determined with the postcoital test

Agglutinin titre (Reciprocal)	Penetration degree 0	1	2	Total
16384	1			1
4096–8192	1			1
1024–2048	3			3
256–512	3		1	4
64–128	3		2	5
16–32	3	1		4
4–8	1	2	1	4
Total	15	3	4	22

Table IV *The Degree of Cervical Mucus Penetration of the Subject's Spermatozoa in Relation to the Sperm Immobilizing Activity of the Corresponding Serum*

Mucus penetration determined with the slide test

Immobilizing activity	Penetration degree			Total
	0	1	2	
<3 h	4	1		5
3–<6 h	6	3	2	11
6–<12 h	2	4		6
≥12 h		7	7	14
Total	12	15	9	36

b. Mucus penetration determined with the capillary-tube test

Immobilizing activity	Penetration degree			Total
	0	1	2	
3 h	5			5
3–<6 h	6	4	1	11
6–<12 h		3	3	6
≥12 h	1	1	12	14
Total	12	8	16	36

Mucus penetration determined with the postcoital test

Immobilizing activity	Penetration degree			Total
	0	1	2	
3 h	4			4
3– 6 h	7	1	1	9
6–<12 h	1	1	1	3
≥12 h	3	1	2	6
Total	15	3	4	22

Table V *The Distribution of the Subjects in the Sterile and Fertile Groups According to the Degree of Mucus Penetration of Their Spermatozoa*

a. Mucus penetration determined with the slide test

Group	Penetration degree			Total
	0	1	2	
Sterile	11	5	5	21
Fertile	1	10	4	15
Total	12	15	9	36

b Mucus penetration determined with the capillary-tube test

Group	Penetration degree			Total
	0	1	2	
Sterile	12	2	7	21
Fertile	0	6	9	15
Total	12	8	16	36

than 6 hours and at 6 or more hours. The incidences of penetration degree 0 using this criterion were very similar to those found on both sides of the critical agglutinin titre stage.

The degree of mucus penetration of the spermatozoa, determined with postcoital tests in relation to the immobilizing activity of the serum is given in Table IV c Of 13 sera with a high immobilizing activity 11 sera were related to a penetration degree of 0 The difference with regard to penetration degree between the groups with low and high sperm immobilizing activity is statistically significant ($X^2 = 3.96$ d.f $= 1$ $p < 0.05$)

Comment The difference between the correlation coefficient -0.76 obtained for the immobilizing activity and the penetration degree with the capillary tube method, and the correlation coefficient -0.55 obtained for agglutinin titre and penetration degree with the same method is statistically almost significant ($p = 0.03$)

Cervical mucus penetration and fertility

The penetration degree of the spermatozoa, determined with the slide method, distributed according to fertility is given in Table V a. It is apparent that the penetration ability was higher in the fertile than in the sterile group. The difference between the distributions is statistically highly significant ($X^2 = 9.34$, d.f. $= 2$, $p < 0.01$).

In the fertile group 14 cases of 15 had a penetration degree of 1 or 2, an incidence for which the 95 per cent confidence interval is 68.1–99.8 per cent. In the sterile group this incidence was lower, 10 of 21, for which the 95 per cent confidence interval is 27.5–70.2 per cent.

In one case of the fertile group a penetration degree of 0 was noted.

Table V b comprises the penetration degree of the spermatozoa, determined with the capillary-tube method, in the fertile and sterile groups. Also with this method there was a statistically highly significant difference ($X^2 = 13.69$, d.f. $= 2$, $p < 0.01$) between the fertile and sterile groups in regard to penetration degree.

In all of the 15 cases of the fertile group the penetration degree was 1 or 2. The 95 per cent confidence interval of this incidence is 78.2–100.0 per cent. As with the slide method, the incidence of penetration at degrees 1 or 2 was lower in the sterile group, ' of 21, the 95 per cent confidence interval of which is 21.8–66.0 per cent.

In no case of the fertile group was a penetration degree of 0 found with the capillary-tube method.

Discussion

The material comprises a group of men with one feature in common, the presence of sperm antibodies in their blood. The level of the sperm antibodies was measured, and the mucus penetration ability of the men's spermatozoa was determined through postcoital tests and with two *in vitro* methods involving determination of the penetration in selected cervical mucus. Thus the existence of a correlation between the level of sperm anti-

Table V *The Distribution of the Subjects in the Sterile and Fertile Groups According to the Degree of Mucus Penetration of Their Spermatozoa*

a. Mucus penetration determined with the slide test

Group	Penetration degree			Total
	0	1	2	
Sterile	11	5	5	21
Fertile	1	10	4	15
Total	12	15	9	36

b. Mucus penetration determined with the capillary tube test

Group	Penetration degree			Total
	0	1	2	
Sterile	12	2	7	21
Fertile	0	6	9	15
Total	17	8	16	36

than 6 hours and at 6 or more hours. The incidences of penetra tion degree 0 using this criterion were very similar to those found on both sides of the critical agglutinin titre stage

The degree of mucus penetration of the spermatozoa determined with postcoital tests, in relation to the immobilizing activity of the serum is given in Table IV c Of 13 sera with a high immobilizing activity 11 sera were related to a penetration degree of 0 The difference with regard to penetration degree between the groups with low and high sperm immobilizing activity is statistically significant ($X^2 = 3\ 96$ d.f $= 1$ $p < 0\ 05$)

Comment The difference between the correlation coefficient $-0\ 76$ obtained for the immobilizing activity and the penetra tion degree with the capillary-tube method, and the correlation coefficient $-0\ 55$ obtained for agglutinin titre and penetration degree with the same method, is statistically almost significant ($p = 0\ 08$)

as regards the immobilizing activity (significant) than the agglutinin titre (almost significant) These facts emphasize the importance of the immobilizing antibodies in comparison to the agglutinating antibodies and point up the desirability of further elaboration and more extensive use of methods for determination of sperm immobilizing antibodies.

The capillary-tube method of Kremer for the determination of the penetration ability of spermatozoa makes it possible to study many ejaculates with the same mucus sample. Kremer used a 70 mm mucus column, but since the cervical canal is seldom more than 30 mm in length, the mucus columns used in the present study were restricted to 30–40 mm. In this way more ejaculates could be tested with the same sample of cervical mucus.

It was noted that the highest correlation between sperm antibodies and penetration degree was achieved when the penetration degree was determined with the capillary-tube method and these results correlated to the immobilizing activity of the serum. Another indication of the reliability of the capillary-tube method was its high prognostic capacity If it is assumed that fertility is incompatible with a penetration degree of 0 there were no prognostic failures among the 15 men in the fertile group The corresponding failure of the slide method was 1 15.

The validity of the postcoital test as a criterion of the penetration ability of spermatozoa is limited by the variation of the quality of the cervical mucus. Yet the relationship between high sperm antibody levels and reduced mucus penetration was also apparent with this method.

There was a statistically significant negative correlation between the level of the sperm agglutinin titre or the concentration of immobilizing activity on one hand and the penetration ability of the spermatozoa on the other The correlation coefficients varied between −0.55 and −0 76 depending on the type of antibody tested and the method used for determination of the penetration degree Considering the fact that the penetration ability of spermatozoa depends on numerous factors it is remarkable that some of these correlations were so high.

Furthermore the fertile and sterile groups of men could be

bodies and penetration degree could be investigated. Finally the sperm antibody levels and the penetration ability of spermatozoa could be analyzed in relation to sterility as some men lived in infertile marriages and others in marriages where the wives had conceived or were recently delivered.

The slightly modified direct sperm agglutination method of Kibrick used in this investigation was discussed in detail in an earlier paper (*Fjällbrant* 1968)

Unfortunately there are no generally accepted methods for accurate determination of the percentage of motile spermatozoa and the degree of motility Sperm immobilizing antibodies have as yet been studied only by a few investigators and the reliability of the methods used seems questionable Generally small quantities of a mixture of sperm serum and complement sealed on a slide have been incubated and the proportion motile/immotile spermatozoa read after intervals When one considers that in addition to immobilization there is also agglutination of spermatozoa it is obvious that the estimation of the proportion motile/immotile spermatozoa, which is always difficult, must be even more difficult with a method which involves counting and judging the motility of agglutinated spermatozoa. The technique used in this investigation eliminates this difficulty as the agglutinates are disrupted before reading The method was standardized by using throughout the investigation spermatozoa from only one donor and an initial proportion of about 70 per cent motile sperm The sperm density however varied somewhat from time to time and the volume of fresh guinea pig serum added was probably greater than necessary Nevertheless an indication of the value of the method is given by the finding that the highest correlation between sperm antibodies and penetration degree was achieved when the immobilizing activity of the sera, determined with this method was correlated to the degree of penetration determined with the capillary tube method. The difference between the coefficient of this correlation and the coefficient for the correlation between sperm agglutinin titres and the penetration degree determined with the same method, was statistically almost significant. Furthermore, the probability of a difference between the fertile and sterile groups was higher

as regards the immobilizing activity (significant) than the agglutinin titre (almost significant) These facts emphasize the importance of the immobilizing antibodies in comparison to the agglutinating antibodies and point up the desirability of further elaboration and more extensive use of methods for determination of sperm immobilizing antibodies.

The capillary-tube method of Kremer for the determination of the penetration ability of spermatozoa makes it possible to study many ejaculates with the same mucus sample. Kremer used a 70 mm mucus column, but since the cervical canal is seldom more than 30 mm in length, the mucus columns used in the present study were restricted to 30–40 mm. In this way more ejaculates could be tested with the same sample of cervical mucus.

It was noted that the highest correlation between sperm antibodies and penetration degree was achieved when the penetration degree was determined with the capillary-tube method and these results correlated to the immobilizing activity of the serum. Another indication of the reliability of the capillary-tube method was its high prognostic capacity If it is assumed that fertility is incompatible with a penetration degree of 0 there were no prognostic failures among the 15 men in the fertile group The corresponding failure of the slide method was 1 15.

The validity of the postcoital test as a criterion of the penetration ability of spermatozoa is limited by the variation of the quality of the cervical mucus. Yet the relationship between high sperm antibody levels and reduced mucus penetration was also apparent with this method.

There was a statistically significant negative correlation between the level of the sperm agglutinin titre or the concentration of immobilizing activity on one hand and the penetration ability of the spermatozoa on the other The correlation coefficients varied between -0.55 and -0.76 depending on the type of antibody tested and the method used for determination of the penetration degree Considering the fact that the penetration ability of spermatozoa depends on numerous factors, it is remarkable that some of these correlations were so high.

Furthermore, the fertile and sterile groups of men could be

bodies and penetration degree could be investigated. Finally the sperm antibody levels and the penetration ability of spermatozoa could be analyzed in relation to sterility, as some men lived in infertile marriages and others in marriages where the wives had conceived or were recently delivered

The slightly modified direct sperm agglutination method of Kibrick used in this investigation was discussed in detail in an earlier paper (*Fjällbrant* 1968)

Unfortunately there are no generally accepted methods for accurate determination of the percentage of motile spermatozoa and the degree of motility Sperm immobilizing antibodies have as yet been studied only by a few investigators, and the reliability of the methods used seems questionable Generally small quantities of a mixture of sperm serum and complement sealed on a slide have been incubated and the proportion motile/immotile spermatozoa read after intervals When one considers that in addition to immobilization there is also agglutination of spermatozoa it is obvious that the estimation of the proportion motile/ *immotile spermatozoa which is always difficult, must be* even more difficult with a method which involves counting and judging the motility of agglutinated spermatozoa. The technique used in this investigation eliminates this difficulty as the agglutinates are disrupted before reading. The method was standardized by using throughout the investigation spermatozoa from only one donor and an initial proportion of about 70 per cent motile sperm The sperm density however varied somewhat from time to time and the volume of fresh guinea pig serum added was probably greater than necessary Nevertheless an indication of the value of the method is given by the finding that the highest correlation between sperm antibodies and penetration degree was achieved when the immobilizing activity of the *sera determined with this method was correlated to the degree* of penetration determined with the capillary tube method. The difference between the coefficient of this correlation and the coefficient for the correlation between sperm agglutinin titres and the penetration degree determined with the same method, was statistically almost significant. Furthermore the probability of a difference between the fertile and sterile groups was higher

as regards the immobilizing activity (significant) than the agglutinin titre (almost significant) These facts emphasize the importance of the immobilizing antibodies in comparison to the agglutinating antibodies and point up the desirability of further elaboration and more extensive use of methods for determination of sperm immobilizing antibodies.

The capillary-tube method of Kremer for the determination of the penetration ability of spermatozoa makes it possible to study many ejaculates with the same mucus sample Kremer used a 70 mm mucus column, but since the cervical canal is seldom more than 30 mm in length, the mucus columns used in the present study were restricted to 30–40 mm. In this way more ejaculates could be tested with the same sample of cervical mucus.

It was noted that the highest correlation between sperm antibodies and penetration degree was achieved when the penetration degree was determined with the capillary-tube method and these results correlated to the immobilizing activity of the serum. Another indication of the reliability of the capillary-tube method was its high prognostic capacity If it is assumed that fertility is incompatible with a penetration degree of 0 there were no prognostic failures among the 15 men in the fertile group The corresponding failure of the slide method was 1 15.

The validity of the postcoital test as a criterion of the penetration ability of spermatozoa is limited by the variation of the quality of the cervical mucus. Yet the relationship between high sperm antibody levels and reduced mucus penetration was also apparent with this method.

There was a statistically significant negative correlation between the level of the sperm agglutinin titre or the concentration of immobilizing activity on one hand and the penetration ability of the spermatozoa on the other The correlation coefficients varied between -0.55 and $-0\ 76$ depending on the type of antibody tested and the method used for determination of the penetration degree Considering the fact that the penetration ability of spermatozoa depends on numerous factors, it is remarkable that some of these correlations were so high.

Furthermore the fertile and sterile groups of men could be

bodies and penetration degree could be investigated Finally the sperm antibody levels and the penetration ability of spermatozoa could be analyzed in relation to sterility as some men lived in infertile marriages and others in marriages where the wives had conceived or were recently delivered.

The slightly modified direct sperm agglutination method of Kibrick used in this investigation was discussed in detail in an earlier paper (*Fjällbrant* 1968)

Unfortunately there are no generally accepted methods for accurate determination of the percentage of motile spermatozoa and the degree of motility Sperm immobilizing antibodies have as yet been studied only by a few investigators and the reliability of the methods used seems questionable Generally small quantities of a mixture of sperm serum and complement sealed on a slide have been incubated and the proportion motile/immotile spermatozoa read after intervals. When one considers that in addition to immobilization there is also agglutination of spermatozoa it is obvious that the estimation of the proportion motile/immotile spermatozoa, which is always difficult, must be even more difficult with a method which involves counting and judging the motility of agglutinated spermatozoa. The technique used in this investigation eliminates this difficulty as the agglutinates are disrupted before reading The method was standardized by using throughout the investigation spermatozoa from only one donor and an initial proportion of about 70 per cent motile sperm. The sperm density however varied somewhat from time to time and the volume of fresh guinea pig serum added was probably greater than necessary Nevertheless an indication of the value of the method is given by the finding that the highest correlation between sperm antibodies and penetration degree was achieved when the immobilizing activity of the sera determined with this method was correlated to the degree of penetration determined with the capillary tube method. The difference between the coefficient of this correlation and the coefficient for the correlation between sperm agglutinin titres and the penetration degree determined with the same method was statistically almost significant. Furthermore the probability of a difference between the fertile and sterile groups was higher

spermatozoa is an important measure in the analysis of sterility. In practice, this determination is, however often difficult to perform under controlled conditions. Thus there is a need for simple laboratory tests, the results of which can be correlated to sperm penetration ability. The investigation shows that the determination of sperm antibodies in blood is such a test. The present results indicate the existence of a correlation between the sperm antibody level in blood and the penetration ability of spermatozoa, but the causality of this relationship has not been demonstrated. Hence it would be of interest to investigate whether sperm antibodies actually cause a reduction of the penetration ability of spermatozoa.

SUMMARY

In 36 men with sperm antibodies of whom 15 lived in fertile and 21 in sterile marriages, the sperm agglutinin titre and sperm immobilizing activity of the serum were determined. The penetration ability of their spermatozoa was investigated with post coital tests and with two methods in which selected cervical mucus was used.

The incidence of high sperm antibody titres was higher in the sterile than in the fertile group. A negative correlation was found between the concentrations of antibodies, especially those with immobilizing activity and the penetration ability of the spermatozoa. The interrelation between penetration ability and fertility was very good.

It is concluded that sperm antibodies at high levels in the blood, particularly immobilizing antibodies, can indicate a reduced penetration ability of spermatozoa and reduced fertility in men. The investigation does not show whether the relationship between the level of sperm antibodies and sperm penetration ability is a causal one.

Acknowledgements

The statistical calculations were revised by Lector E. Carlström, the Department of Statistics, University of Göteborg. Miss Y

differentiated in relation to the levels (high and low) of the agglutinating and immobilizing antibodies In the instance of the sperm immobilizing antibodies the group difference was statistically significant.

As might be expected there was a very good interrelation between fertility and the penetration ability of the spermatozoa. The difference between the sterile and fertile groups as regards penetration degree was statistically highly significant. When judging the interrelation between the penetration degree of the spermatozoa and fertility however it may be more relevant to consider only the fertile group because this is less influenced by female infertile factors than the sterile group. In the fertile group accordingly a penetration degree of 1 of 2 was found in 14 of 15 cases with the slide method and in all the 15 cases with the capillary-tube method.—Parenthetically it should be pointed out that not only penetration degree 2 (normal penetration) but also degree 1 (reduced propagation) were found in the fertile group

According to experience this relationship between penetration ability of spermatozoa and fertility is a causal one Independent of the nature of the correlation between the sperm antibody level and the mucus penetration degree found in this investigation it is clear because there is a significant and negative correlation between the sperm antibody level of the blood and the degree of cervical mucus penetration by spermatozoa, that a high concentration of sperm antibodies found in blood from men can be interpreted to indicate impaired fertility As regards the prognostic possibilities it could be stated that the mucus penetration ability of spermatozoa was reduced rather abruptly at a certain stage of the sperm antibody level. The sperm immobilizing activity of serum gave a higher correlation to the mucus penetration degree than the agglutinin titre and therefore seems preferable for prognostic use. Of the methods adopted for determination of the penetration ability of spermatozoa the capillary-tube test showed the greatest prognostic capacity with regard to fertility

Experience has shown and the results of the present investigation indicate that determination of the penetration ability of

spermatozoa is an important measure in the analysis of sterility In practice this determination is, however often difficult to perform under controlled conditions. Thus there is a need for simple laboratory tests, the results of which can be correlated to sperm penetration ability The investigation shows that the determination of sperm antibodies in blood is such a test. The present results indicate the existence of a correlation between the sperm antibody level in blood and the penetration ability of spermatozoa, but the causality of this relationship has not been demonstrated. Hence it would be of interest to investigate whether sperm antibodies actually cause a reduction of the penetration ability of spermatozoa.

SUMMARY

In 36 men with sperm antibodies of whom 15 lived in fertile and 21 in sterile marriages the sperm agglutinin titre and sperm immobilizing activity of the serum were determined. The penetration ability of their spermatozoa was investigated with post coital tests and with two methods in which selected cervical mucus was used.

The incidence of high sperm antibody titres was higher in the sterile than in the fertile group. A negative correlation was found between the concentrations of antibodies, especially those with immobilizing activity and the penetration ability of the spermatozoa. The interrelation between penetration ability and fertility was very good.

It is concluded that sperm antibodies at high levels in the blood, particularly immobilizing antibodies, can indicate a reduced penetration ability of spermatozoa and reduced fertility in men. The investigation does not show whether the relationship between the level of sperm antibodies and sperm penetration ability is a causal one

Acknowledgements

The statistical calculations were revised by Lector E. Carlström, the Department of Statistics, University of Göteborg. Miss Y

Steen performed as technical assistant. The English text was revised by Translator C Wadsworth. The investigation was supported by grants from The Medical Society of Goteborg and The Faculty of Medicine, University of Goteborg

REFERENCES

Fjällbrant B. Immunoagglutination of sperm in cases of sterility Acta obstet. gynec. scand. *44* 474 1965

– Sperm agglutinins in sterile and fertile men. Acta obstet. gynec. scand. *47* 89 1968

Kibrick S Belding, D L. and *Merrill B* Methods for the detection of antibodies against mammalian spermatozoa. II. A gelatin agglutination test. Fertil. and Steril. *3* 430 1952

Kremer J A simple sperm penetration test. Int. J Fertil. *10* 209 1965

Rümke P and *Hellinga G* Autoantibodies against spermatozoa in sterile men. Amer J clin. Path. *32* 357 1959

Wilson L Sperm agglutinins in human semen and blood. Proc. Soc. exp. Biol. (N Y) *85* 652, 1954

– Sperm agglutination due to autoantibodies. A new cause of sterility Fertil and Steril *7* 262, 1956

Received on July 14 1967

Nielsen, O Vagn, Acta obst. et gynec. scandinav. 47:18, 1968
From the Department of Surgery St. Elisabeth Hospital (J Balslev Jørgensen M.D. and H Munkvast M.D.) Copenhagen Denmark

OVARIAN TUMOURS IN CHILDREN

Report of a Rare Case a Thecoma in a 12-year-old Girl

BY

O VAGN NIELSEN

Ovarian tumours are rare in children and adolescent girls. According to *Kaplan* and *Hayem* (1963) these tumours make up only 1 per cent of all tumours affecting children under 15 years of age

The first survey on ovarian tumours in children was published by *Wiel* (1905) who had collected 61 cases *Downes* (1921) published another 26 cases and *Loeb* and *Levy* (1932) 35 cases. *Witzberger* and *Agerty* (1937) collected yet another 64 cases, which brought the number of cases up to a total of 186 All 186 patients were under 10 years and were not yet menstruating

Groeber (1963) collected 14 series of ovarian tumours in children, which had been published since 1937 including his own series of 13 cases In his paper the age limit was somewhat extended, most of the series including patients up to and including the age of 15 Thus, in a number of these cases the tumours had appeared after the menarche. However *Dente* (1960) has reported 36 cases, *Forshall* (1960) 21 cases, *Boles Hardacre* and *Newton* (1961) 16 cases and *Reis* (1962) 25 cases—a total of 98 tumours—which had definitely occurred before the menarche. The patients of *Radman*'s (1960) series of 27 children and young girls (also included in Groeber's review) ranged in age from 7–18 years)

Furthermore *Sjövall* (1944) and *Gagner* and *Sjövall* (1949) collected 43 patients (all under 15) with ovarian tumours. *Of* these cases 39 were found upon perusal of the files of the Pathological Institute of the Lund University for the previous 50 years.

In 1963 *Thatcher* published a series of 48 children aged 0–14 years

Adams and *Kaufman* (1962) reported 13 cases of ovarian tu mours occurring before the menarche

Abell Johnson and *Holt*. (1965) have reported on 186 tumours in 182 patients but they included girls of 19 years and under Only 35 of their patients had not begun to menstruate

Lastly *Irons* (1966) reported a series of 7 girls of premenstrual age

If all these series are added up about 770 ovarian tumours in children and young girls are on record but between 200 and 300 of these tumours occurred after the menarche In addition to these series of ovarian tumours a number of individual cases have been reported usually of the more uncommon types e g by *Zemke* and *Herrell* (1943) *Limburg* (1947) *Gordon* and *Marvin* (1951) *Knauss Campos* and *Rose* (1953) *Neigus* (1957) *Green* (1960) *Faber* (1962) and *Steckel* (1965)

Pathology The distribution of the various types of tumour in the published series is fairly uniform

The most common type is the benign *teratoma* or *dermoid cyst* *Witzberger* and *Agerty* (1937) found 31 per cent teratomas among 186 cases *Gagner* and *Sjövall* (1949) 35 per cent out of 43 tumours Among 263 cases *Groeber* (1963) found 131 (50 per cent) to be benign cystic teratomas 21 (8 per cent) solid teratomas and 7 (3 per cent) malignant teratomas *Adams* (1962) found 70 per cent teratomas and *Thatcher* (1963) 22 9 per cent Out of *Abell Johnson* and *Holt*'s (1965) 182 patients (aged 0–19) 50 per cent had tumours of the teratoma type *vi* 38 per cent mature teratomas (dermoid cysts) 7 per cent partially dif ferentiated teratomas and 5 per cent embryonic teratomas (car cinomas) If only the premenstrual cases (35 children) are con sidered, the named types of teratoma occurred in 45 12 and 16 per cent respectively

Second in frequency are *follicular cysts* simple or multilocular In their collected material of 186 patients *Witzberger* and *Agerty* (1937) found 60 or 32 per cent to have cysts *Groeber* (1963) reported that 16 per cent, or 43 of 263 patients had cysts and *Thatcher* (1963) that 47.9 per cent had follicular cysts.

Other types of ovarian tumours in children are far more uncommon. It is characteristic that in the early series a fairly high percentage of *carcinoma* and *sarcoma* was reported. For instance *Wiel* (1905) reported 25 per cent sarcomas and 8.3 per cent carcinomas, *Downes* (1921) 61.5 per cent carcinomas + sarcomas *Loeb* and *Levy* (1932) 14 7 per cent carcinomas and 11 7 per cent sarcomas and lastly *Würzberger* and *Agerty* (1937) stated that combined malignancies occurred in 38 per cent of their 186 patients under 10 years of age. The differentiation of the various types of tumour was not as accurate as it is to-day so that some of the above-mentioned combined malignancies must have been granulosa-cell tumours and dysgerminomas—which are not even mentioned among the 186 patients

In *Groeber*'s large material (1963) *granulosa-cell tumours* made up 5 per cent, *dysgerminomas* 3 per cent, *serous cystadenomas* 4 per cent, and *pseudomucinous cystadenomas* 3 per cent. The carcinoma and sarcoma type is considerably more uncommon than previously reported. Thus, *Groeber* reported 5 per cent adenocarcinomas 2 per cent embryonic carcinomas, and 0 4 per cent pseudomucinous cystadenocarcinomas. As for sarcomas there were only 2 cases out of 263 i.e 0.8 per cent.

Gagner and *Sjövall* (1949) found a fairly high percentage of dysgerminomas viz 14 per cent (6 out of 43 patients)

The share of granulosa-cell tumours has been very constant in the more recent analyses. *Morris* and *Scully* (1958) found that out of roughly 1000 cases of granulosa-cell tumours on record, at that time 5 per cent had occurred before puberty

In these numerous series of ovarian tumours in children totalling about 770 patients a few types of tumour are not even represented.

Arrhenoblastoma was not believed to occur before puberty (*Dargeon* 1960) But this virilizing tumour does occur in children, for *Novak* and *Long* (1965) on perusal of "The Ovarian Tumor Registry which was started in 1942, found that among 111 patients with arrhenoblastoma 3 were in the prepubertal age group i.e 8 6 and 2 years.

Thecoma is also not represented among these many series of ovarian tumours in children. However a few isolated cases have

been reported, by *Geist* and *Gaines* (1938) *Limburg* (1947) *Gordon* and *Marvin* (1951) *Knauss* *Campos* and *Rose* (1953) and *Faber* (1962) Among these cases however *Limburg* s as well as *Knauss* were not pure thecomas, but mixed tumours consisting of granulosa-cell as well as theca-cell elements.

Malignancy Statements about the malignancy of the ovarian tumours differ widely Among 186 tumours *Wittberger* and *Agerty* (1937) found malignancy in 38 per cent. In *Gagner* and *Sjövall*'s (1949) series of 43 tumours 16 were malignant (37 per cent) *Darte* (1960) reported 5 malignant cases out of 36 or 14 per cent, *Groeber* (1963) 19 per cent out of 263 cases *Adams* (1962) 30 per cent malignancy (among 13 children) *Thatcher* (1963) only 10.5 per cent malignancy *viz.* 5 out of 48 Lastly among *Abell* *Johnson* and *Holtz* s (1965) 186 tumours 41 or 23 per cent were malignant. When considering only the 35 premenstrual cases of the last mentioned series the malignancy rate is far higher *viz.* 43 per cent or 15 cases

Age incidence All authors agree that in very young children (under 5 years) ovarian tumours are extremely rare and that a definite increase in the incidence occurs around and immediately after puberty Analysing the age distribution of his patients aged 0–14 years *Groeber* (1963) found that most cases occurred in the 10–14-year group However this did not apply to the granulosa-cell tumours of which only 12 per cent occurred in the age range 10–14 years while 42 per cent occurred from 0–4 years and the remaining 46 per cent in the age range 5–9 years

Gagner and *Sjövall* (1949) found the incidence of ovarian tumours to increase towards puberty In their series of 43 children the incidence was as follows 0–5 years 5 (11 6 per cent) 6–10 years 7 (16 3 per cent) 11–15 years 31 (72 1 per cent)

In *Abell* *Johnson* and *Holtz* s (1965) large series of 182 patients aged 0–19 years only 35 or 19 per cent were in the premenstrual age group

Diagnosis. The diagnosis of ovarian cysts or tumours in children is often very difficult, and mistakes were made in several of the reported cases.

The most common symptom is abdominal pain—chronic pains due to pressure of the tumour upon its surroundings and acute pain frequently caused by torsion of the pedicle of the cyst or tumour Out of *Butt* s (1955) 25 cases 18 or 72 per cent had abdominal pain. Pain was also present in 70 per cent (25 out of 36 cases) in *Darte* s (1960) in 54 per cent (7 patients out of 13) in *Adams* and *Kaufman* s (1962) in 15 (60 per cent) out of 25 cases in *Reis*' (1962) and in 37 out of 48 (77 per cent) in *Thatcher* s (1963) series.

In the cases with acute pain the condition is most often misdiagnosed as acute appendicitis. For instance in 7 out of *Loeb* and *Levy* s (1932) 13 cases this was the preoperative diagnosis. Out of *Gagner* and *Sjövall*'s (1949) 43 patients 25 were admitted as acute abdominal cases, and in 11 of them the preoperative diagnosis was acute appendicitis. Moreover *Thatcher* (1963) had the diagnosis of acute appendicitis in 43 per cent, or 21 of 48 children.

The most common sign is an abdominal mass. Owing to the small space in the true pelvis of children, the ovarian tumours will soon grow upwards and present themselves as abdominal, not pelvic masses. However in many cases the tumour is palpable through the rectum.

Owing to the abdominal situation of the mass, ovarian tumours in children present many differential diagnostic possibilities. According to *Arey* (1963) the most common abdominal tumours in children are neuroblastoma and Wilms tumour but a number of other possibilities must be taken into consideration mesenteric cysts, retroperitoneal tumours, hepatic tumours urachal cysts, polycystic kidneys hydronephrosis, bladder stones, retention of urine ascites haematometra haematosalpinx, and pregnancy Several of these possibilities may often be excluded by preoperative investigations, such as *i v* pyelography X-ray examination of the colon but nevertheless the preoperative diagnosis is often wrong. *Thatcher* (1963) stated that in 36 of his 48 cases the preoperative diagnosis was wrong

In cases with abdominal pain, an abdominal mass, and perhaps elevation of temperature the condition is most often diagnosed as an appendix abscess.

been reported by *Geist* and *Gaines* (1938) *Limburg* (1947) *Gordon* and *Marvin* (1951) *Knauss Campos* and *Rose* (1953) and *Faber* (1962) Among these cases however *Limburg's* as well as *Knauss* were not pure thecomas but mixed tumours con sisting of granulosa-cell as well as theca-cell elements.

Malignancy Statements about the malignancy of the ovarian tu mours differ widely Among 186 tumours *Witzberger* and *Agerty* (1937) found malignancy in 38 per cent In *Gagner* and *Sjövall's* (1949) series of 43 tumours 16 were malignant (37 per cent) *Darte* (1960) reported 5 malignant cases out of 36 or 14 per cent, *Groeber* (1963) 19 per cent out of 263 cases *Adams* (1962) 30 per cent malignancy (among 13 children) *Thatcher* (1963) only 10.5 per cent malignancy *vi* 5 out of 48 Lastly among *Abell Johnson* and *Holt* s (1965) 186 tumours 41 or 23 per cent were malignant. When considering only the 35 premenstrual cases of the last-mentioned series the malignancy rate is far higher *vi* 43 per cent or 15 cases.

Age incidence All authors agree that in very young children (under 5 years) ovarian tumours are extremely rare and that a definite increase in the incidence occurs around and immediately after puberty Analysing the age distribution of his patients aged 0–14 years *Groeber* (1963) found that most cases occurred in the 10–14-year group However this did not apply to the granu-losa-cell tumours of which only 12 per cent occurred in the age range 10–14 years while 42 per cent occurred from 0–4 years and the remaining 46 per cent in the age range 5–9 years

Gagner and *Sjövall* (1949) found the incidence of ovarian tumours to increase towards puberty In their series of 43 chil dren the incidence was as follows 0–5 years 5 (11 6 per cent) 6–10 years 7 (16 3 per cent) 11–15 years 31 (72 1 per cent)

In *Abell Johnson* and *Holt*'s (1965) large series of 182 pa tients aged 0–19 years only 35 or 19 per cent were in the pre-menstrual age group

Diagnosis The diagnosis of ovarian cysts or tumours in children is often very difficult, and mistakes were made in several of the reported cases.

be mentioned that according to *Diddle* 926 cases of granulosa-cell tumour had been reported, 52 of which, or 6 per cent, occurred in the age group 0–10 years.

According to *Morris* and *Scully* (1958) more than 1000 granulosa-cell tumours and 300 theca-cell tumours are on record. Among the granulosa-cell tumours 5 per cent have occurred be fore puberty 55 per cent during the child-bearing age and 40 per cent after the menopause. Among the thecomas, 3 are said to have been reported before puberty (1 per cent) while 60 per cent have occurred after the menopause. With regard to the malignancy of the granulosa-cell tumour group *Morris* and *Scully* (1958) state that, in general, thecomas are benign, although 10 malignant cases have been reported. However thecomas must be considered relatively benign, compared with granulosa-cell tumours. Out of 926 granulosa-cell tumours there were recurrences of 92 (10 per cent) and out of 263 thecomas there were recur rences of only 4 (about 1.5 per cent) (*Diddle* 1952) *Novak* and *Woodruff* (1962) give a different opinion, finding, in an analysis of a very large series (including The Ovarian Tumor Registry) with a long term (though still insufficient) follow-up about 25 per cent recurrences in the entire group Thus the prognosis for these patients (including those with thecomas) appears to be somewhat poorer than previously assumed. Furthermore the usual 5-year follow up period must be considered too brief as recurrences may appear several years after the first operation—even up to about 20 years. *TeLinde* (1962) also considers these tumours to be not quite as benign as previously assumed, as re currences may appear after many years.

Only a very few cases of theca-cell tumours in children have been reported (so far)

Geist and *Gaines* (1938) mentioned, among 6 cases of ovarian thecoma a case in a 16-year-old girl. Her complaints were primary amenorrhoea abdominal discomfort, and furthermore hoar seness slight hirsutism, and enlargement of the clitoris to 3 times the normal size. Operation revealed a right-sided ovarian tumour (8×5×5.5 cm) encapsulated, and greyish-yellow which, on histological examination, proved to be an ovarian thecoma.

Ovarian Thecoma This type of ovarian tumour which is relatively uncommon in adults and extremely rare in children, was described for the first time in 1932 by *Löffler* and *Priesel* who called it fibroma theco-cellulare xanthomatodes ovarii"

In classifications of ovarian tumours the thecomas are assigned to the group of granulosa-cell tumours—which comprises granulosa-cell tumours theca-cell *tumours* (thecoma) and luteinized granulosa-cell tumour (*Teilum* 1952)

Granulosa as well as theca cells are derived from the ovarian mesenchyme As *Novak* and *Woodruff* say (1962) "It is from the ovarian mesenchyme that both granulosa and theca cells are developed and hence it is not surprising that the feminizing tumors may develop either epithelial (granulosal) or connective tissue (thecal) morphologic characteristics Moreover in many tumors one finds a mixture of the two elements. It seems logical, *therefore to look to the common progranulosal* and prothecal mesenchyme as the source of origin of these neoplasms.

Macroscopically the theca-cell tumours are usually enclosed in a distinct capsule firm and elastic in consistency and show on their surface as well as on their cut surface a yellowish white appearance The cut surface often shows small cystic and/or necrotic areas

Microscopically they exhibit an extremely uniform appearance characterized according to *Novak* and *Woodruff* (1962) by the presence of broad spindle cells epithelioid of appearance distributed in an irregular interlacing manner throughout the tumour separated by varying sized bands of connective tissue and often hyaline plaques

Fat staining often affords great help in cases of diagnostic doubt as the thecomas contain intra as well as extracellular fat droplets which give the tumour its yellowish colour

Since the first description in 1932 a number of cases have been reported. Reviewing the literature up to 1944 *McGoldrick* and *Lapp* found 82 cases of thecoma 65 per cent of which occurred after the menopause 35 per cent between puberty and the menopause while no case had been reported before puberty *Diddle* (1952) found 263 thecomas in the literature including 1 case in a girl aged 16 (*Geist* and *Gaines* 1938) By comparison it may

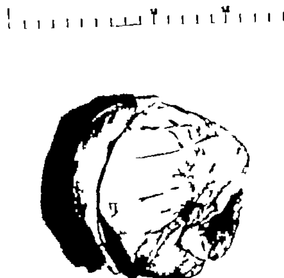


Fig. 1 Macroscopic appearance of the tumour. Size 12×9×13 cm.

to hospital and had, on the whole, always been in good health. She was not yet menstruating.

On physical examination, her appearance and her development were normal for her age. Perhaps the breasts were rather well-developed, and there was an early growth of axillary and pubic hair but not in excess of what is normal for 12-year-old girl.

Palpation of the abdomen revealed mass extending from the symphysis up to the umbilicus. On rectal examination there was a small, movable cervix in which no orifice could be palpated, and which appeared to merge into round, smooth, non-tender mass (the uterus) the size of coconut. There was no vaginal atresia, and pregnancy test was negative.

As the findings were at first interpreted as representing haematometra due to cervical atresia, the hymen was excised, giving access to normal-looking, virginal portio vaginalis with an orifice which admitted a 5 cm probe. Thus, the diagnosis of haematometra could not be maintained. Bimanual examination now gave the impression of normal small uterus above which there was mobile hard tumour as large as an infant head. Hystero-salpingography showed rather long uterine cavity compressed by the tumour from the outside. The uterine cavity was pressed to the right.

Now the most likely diagnosis was an ovarian tumour. Laparotomy revealed mobile, smooth tumour the size of an infant head, arising in the

Limburg (1947) described a 2 year-old girl with an abdominal tumour as large as an infant s head and evidence of precocious puberty such as hypertrophy of the breasts and a sero-sanguineous vaginal discharge Operation disclosed a left-sided ovarian tumour (17×11×9 cm) which was whitish cystic and smooth walled. Microscopic examination showed a mixed tumour composed of theca-cell as well as granulosa-cell elements *i.e* not a pure thecoma

Reviewing the literature of the preceding 20 years *Gordon* and *Marvin* (1951) found no case of ovarian thecoma in premenstrual children These authors mention *Limburg s* case (from 1947) but they do not consider it a real thecoma as it was a mixed tumour Their own case was a girl aged 1 year 8 days with distended abdomen and precocious puberty At operation a tumour (9 cm in diameter) was removed from the left ovary Microscopic examination showed this to be an ovarian thecoma

Knauss Campos and *Rose* (1953) have reported a left-sided ovarian tumour (15×10×8 cm) in a 9 year-old girl The patient also had ascites and right sided hydrothorax, *i e* Meigs s syndrome. Microscopic examination revealed that the tumour consisted of theca-cell as well as granulosa-cell elements *i e* mixed tumour

Pedowitz Felmus and *MacLles* (1955) described a child of 14 months with precocious puberty and left-sided ovarian tumour (17×10 cm) The histological report was A pure thecoma with several scattered areas of granulosa-cell rests

Faber (1962) reported the case of a girl, aged 4 years with Meigs syndrome and bilateral ovarian tumours measuring on the right 10×16 cm and on the left 8×10 cm Bilateral salpingo-oophorectomy was carried out, and microscopical examination showed the tumours to be thecomas There were no signs of increased hormone production

Only 3 of the 6 above cases appear to have been pure thecomas.

Case Report

(K. 2265/65–66) A girl aged 12 years was admitted as the school doctor had found an abdominal mass (enlargement of the uterus?) corresponding to a 4-5-month pregnancy The patient had never previously been admitted



Fig 1 Macroscopic appearance of the tumour Size 12×9×13 cm.

to hospital and had, on the whole always been in good health. She was not yet menstruating

On physical examination, her appearance and her development were normal for her age Perhaps the breasts were rather well-developed, and there was an early growth of axillary and pubic hair but not in excess of what is normal for 12-year-old girl.

Palpation of the abdomen revealed mass extending from the symphysis up to the umbilicus. On rectal examination there was a small, movable cervix in which no orifice could be palpated, and which appeared to merge into round, smooth, non-tender mass (the uterus?) the size of coconut. There was no vaginal atresia, and pregnancy test was negative.

As the findings were at first interpreted as representing haematometra due to cervical atresia, the hymen was excised giving access to normal-looking, vaginal portio vaginalis with an orifice which admitted 5 cm probe Thus the diagnosis of haematometra could not be maintained Bimanual examination now gave the impression of normal small uterus above which there was mobile hard tumour as large as an infant head Hystero-salpingography showed rather long uterine cavity compressed by the tumour from the outside The uterine cavity was pressed to the right.

Now the most likely diagnosis was an ovarian tumour Laparotomy revealed mobile smooth tumour the size of an infant' head, arising in the

Limburg (1947) described a 2 year-old girl with an abdominal tumour as large as an infant s head and evidence of precocious puberty such as hypertrophy of the breasts and a sero-sanguineous vaginal discharge Operation disclosed a left sided ovarian tumour (17×11×9 cm) which was whitish cystic, and smooth-walled. Microscopic examination showed a mixed tumour composed of theca-cell as well as granulosa-cell elements, *i.e* not a pure thecoma

Reviewing the literature of the preceding 20 years *Gordon* and *Marvin* (1951) found no case of ovarian thecoma in premenstrual children These authors mention *Limburg* s case (from 1947) but they do not consider it a real thecoma as it was a mixed tumour Their own case was a girl aged 1 year 8 days with distended abdomen and precocious puberty At operation a tumour (9 cm in diameter) was removed from the left ovary Microscopic examination showed this to be an ovarian thecoma

Knauss Campos and *Rose* (1953) have reported a left-sided ovarian tumour (15×10×8 cm) in a 9 year-old girl The patient also had ascites and right sided hydrothorax *i.e* Meigs s syndrome. Microscopic examination revealed that the tumour consisted of theca-cell as well as granulosa-cell elements *i e* mixed tumour

Pedowitz Felmus and *Mackles* (1955) described a child of 14 months with precocious puberty and left sided ovarian tumour (17×10 cm) The histological report was A pure thecoma with several scattered areas of granulosa-cell rests

Faber (1962) reported the case of a girl aged 4 years with Meigs syndrome and bilateral ovarian tumours measuring on the right 10×16 cm and on the left 8×10 cm Bilateral salpingo-oophorectomy was carried out and microscopical examination showed the tumours to be thecomas There were no signs of increased hormone production

Only 3 of the 6 above cases appear to have been pure thecomas.

Case Report

(K. 2265/65-66) A girl, aged 12 years was admitted as the school doctor had found an abdominal mass (enlargement of the uterus?) corresponding to a 4-5-month pregnancy The patient had never previously been admitted

2

3

Figs 2 and 3 Microscopic appearances Typical thecoma

left ovary The right ovary was entirely normal. Left salpingo-oophorectomy was carried out.

The postoperative course was uncomplicated, and the girl was discharged 9 days after the operation.

The removed tumour (Fig. 1) weighed 900 g and measured 12×9×13 cm) It was entirely encapsulated firm and elastic in consistency and yellowish-white on the surface as well as on the cut surface In a few areas there was necrotic tissue

Histological examination (Figs. 2 and 3) showed tumour tissue of completely uniform structure, consisting of irregular bundles and strands of spindle-shaped cells and in places a few rather more angular cells. In places there were strands of connective-tissue but there were no granulosa-cell elements and no signs of malignancy The diagnosis was ovarian thecoma.

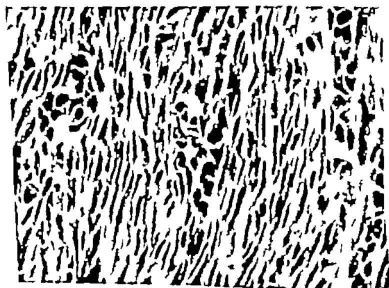
Discussion

It is apparent from the above review of the literature that ovarian tumours in children are relatively uncommon, and frequently difficult to diagnose It must be emphasized that in cases of abdominal pain and/or an abdominal mass in a girl the possibility of an ovarian tumour should be considered. However the diagnosis is frequently only made at exploratory laparotomy

The treatment of ovarian tumours in children is surgical and the operation should be performed as soon as the diagnosis has been made in order to avoid complications such as twisting of the pedicle or rupture and also because of the risk of malignancy True the reported rate of malignancy has differed widely from 10–60 per cent, but no doubt an average risk of about 25 per cent should be expected It is only in cases of benign cysts with preserved normal ovarian tissue that the operation can be restricted to resection of the cyst, with reconstruction of the remaining ovarian tissue In the case of benign solid tumours whose capsule is completely intact, the procedure may be merely salpingo-oophorectomy resecting the uterine cornu But, in malignant cases where the tumour has penetrated the capsule it is necessary to carry out total hysterectomy and bilateral salpingo-oophorectomy

A thecoma of the ovary must be considered extremely rare in premenstrual girls. Of the 6 cases on record only 3 have been pure thecomas without admixture of granulosa-cell elements

2



3

Figs 2 and 3 Microscopic appearances. Typical thecoma

Two of the reported cases (*Knauss Campos* and *Rose s* (1953) and *Faber s* (1962)) have been associated with ascites and hydrothorax *i e* Meigs syndrome. This syndrome is not entirely uncommon in the presence of thecomas in adults. For instance, *Rubin Noval* and *Squire* (1944) found that out of 23 patients with thecoma 7 (30 per cent) had ascites and one hydrothorax.

Three out of the 6 reported cases of which one was definitely a pure thecoma also had precocious puberty These patients were 2 years 1 year 8 days and 14 months of age The granulosa-cell tumour group— feminizing mesenchymomas — and especially the granulosa-cell tumours themselves are the group of ovarian tumours which are most often responsible for precocious puberty *Pedowitz Felmus* and *Mackles* (1955) state that 62 cases of granulosa-cell tumours and 3 thecomas (the 3 mentioned above) associated with precocious puberty are on record. Out of a total of 85 ovarian tumours in children associated with precocious puberty 62 have been granulosa-cell tumours 3 thecomas, 12 teratomas 5 follicular cysts and 3 dysgerminomas.

There has been no report of malignant thecoma in children.

SUMMARY

After reviewing the literature on ovarian tumours in children the pathology of the tumours diagnostic considerations malignancy and treatment the author adds a case of ovarian thecoma in a premenstrual girl aged 12 years. The tumour proved to be a pure thecoma The patient did not show signs of precocious puberty Meigs syndrome or malignancy

REFERENCES

Abell M R., Johnson V J and *Holtz F* Amer J Obst. and Gynec *92* 1059 1965

Adams B D and *Kaufman R H* Southern Med. Journ *55* 801 1962

Arey J B. Pediatric Clin. of North America 665 1963

Biggart J H and *Macafee C H G* J Obst. and Gynec. of The British Empire *62* 829 1955

Boles E. T Hardacre J M and *Newton W A.* Arch of Surg *83* 590 1961

Burt J A. Amer J Obst. and Gynec. *69* 833 1955

Dargeon, H. W. Pediatrics 3 773 1949
Dart J M M Clin. Obst. and Gynec. 3 187 1960
Diddle A. W. Cancer 5 215 1952
Downes, W A., J Amer Med. Ass. 76 443 1921
Faber H K., J of Pediat. 61 769 1962
Forshall L, Arch Dis. Childhood 35 17 1960
Gegner S. and Sjövall A., Acta Obst. et Gynec. Scand. 28 110 1949
Gerr S H and Gaines J A., Amer J Obst. and Gynec. 35 39 1938
Gordon V H and Morris, H N J of Pediat. 39 133 1951
Green, G H Amer J Obst. and Gynec. 79 999 1960
Groeber W R. Amer J. Obst. and Gynec. 86 1027 1963
Irons, G B Hoge R. H and Salzberg, A. M Clin. Pediat. 5 151 1966
Kaplan M. and Hayem F Pediatri 18 177 1963
Kneass, W E. Campos, J and Rose W J. Pediat. 43 88 1953
Limburg, H Ztschr f Geburtsh. u. Gynäk. 128 186 1947
Loeb M. J and Levy W Arch. Pediat 49 651 1932
Löffler E and Priesel A., Beitr z. path. Anat u. allg. Path. 90 199 1932
McGoldrick J L. and Lapp W A Amer J Obst. and Gynec. 48 409 1944
Morris J M and Scully R. E., Endocrine Pathology of the Ovary C V Mosby Company St. Louis, 1958
Novak E. R and Long, J H Amer J Obst. and Gynec 92 1082, 1965
Novak E and Novak E R Gynecologic and Obstetric Pathology W B. Saunders Company 1958
Novak E R and Woodruff R. D Novak Gynecologic and Obstetric Pathology W B Saunders Company 1962
Pedowitz P Felmus, L B. and Mackles A Obst. and Gyn. Surv 10 633 1955
Radman H M and Korman W Amer J Obst and Gynec. 79 989 1960
Reis R L and Knoop C E J of Pediat 60 96, 1962
Ruth I C Novak J and Squire J J Amer J Obst and Gynec. 48, 601 1944
Sjövall A Acta Pathol et Microbiolog. Scand. 54 183 1944
Seckel R J Hendrix E L and Bigelow R R Obst. and Gynec 25 249 1965
Teilum G Acta Obst. et Gynec Scand 31 292, 1952
Te Linde R W Operative Gynecology J B Lippincott Company 1962
Thatcher D S Surg Gynec and Obst. 117 477 1963
Wiel H I The Johns Hopkins Hosp. Bull 16 102, 1905
Wittenberger C M and Agerty H A., Arch. of Pediat 54 339 1937
Zemke E E and Herrell, W E Amer J Obst. and Gynec. 41 704 1941

Received on April 11 1967

Sandholm, Lars-Erik Acta obstet et gynec scandinav 47 [illegible], 1968

From the Department of Obstetrics and Gynaecology (Prof Stig Kullander)
Malmö General Hospital University of Lund Malmö Sweden

VARIATIONS IN THE MILK EJECTION REFLEX IN WOMEN DURING THE EARLY STAGES OF LACTATION

BY

LARS-ERIK SANDHOLM

The milk ejection or "let-down" reflex in animals and in human beings has long been known

Ott and *Scott* (1910) showed that the milk ejection in lactating goats could be stimulated by an extract from the posterior pituitary

Gaines (1915) underlined the difference between the secretion and the ejection of milk Later experiments with purified posterior pituitary extract have shown that milk ejection is elicited mainly by oxytocin which causes contraction of the myo-epithelial cells around the glandular alveoli in the breasts. This contraction expresses the milk into the lactiferous ducts and sinuses, whence it can be removed by suckling or milking (*Richardson* 1949 *Cross* 1961) The pressure in the mammary gland is dependent on the amount of milk in the breast and the contractions of the myo-epithelial cells

In previous investigations of milk ejection in women interest was confined to the appearance of the milk on the mamilla (*Beller et al* 1958 *Douglas et al* 1957 *Nickerson* 1954)

Cross and *Harris* (1952) devised a method for measuring the intramammary pressure in rabbits They introduced a fine cannula or plastic catheter into one of the excretory ducts of the mamilla and recorded the intramammary pressure with a transducer and an amplifier The method permits the recording of responses to intravenous doses of oxytocin down to 0 5 mU The technique has been refined by injection of the test solution

into one of the regional arteries of the mammary gland (*Fitz patrick* 1961)

Several species have since been studied with this cannulation technique (*Cross* and *van Dyke* 1955 *Cross* 1955 *Berde* 1957 and others)

According to the general consensus of opinion, oxytocin is synthetized in neurosecretory cells in the diencephalon, whence it is transported by neurosecretion to the pituitary where it is stored.

On nervous stimulation of the hypothalamus by impulses from the mamilla, for example the hormone is released from the hypophysis into the bloodstream (*Cross* 1961 *Denamur* 1965) The release of oxytocin may be accompanied by secretion of vasopressin. It appears that in human beings oxytocin and vasopressin can be secreted separately (*Gaitan et al.*, 1964 *Cobo et al.* 1965)

The intramammary pressure (IMP) has been measured in women after cannulation of one or more lactiferous ducts of the mamilla (*Sica Blanco et al.* 1960)

Pressure recordings have been made during labour delivery and lactation. A pressure curve of the type seen during lactation can be induced by intravenous infusion of oxytocin in a dose of 4–8 mU per minute Synthetic oxytocin (Syntocinon®) has proved to be 8 to 10 times more effective than Pitressin® or arginin vasopressin (*Sala et al.* 1960)

Friedman (1960) described another method for measuring the intramammary pressure According to this method a perspex cylinder is placed over the mamilla and held there with the aid of a suction cup. The cylinder is connected to the transducer by means of a tube and the system is filled with fluid. With this technique the values obtained are only one tenth as high as those recorded with cannulation. The recordings obtained by the two methods show the same type of fluctuation of the pressure

The milk ejection reflex can be inhibited by emotional factors. This has been shown in guineapigs (*Chaudhury et al.* 1961) and in women (*Newton* and *Newton* 1948)

Adrenalin inhibits milk ejection by its peripheral action (*Cross* 1953) The inhibition may be due to vascular contraction with a consequent fall in the concentration of oxytocin around the myoepithelial cells.

Sandholm, Lars-Erik, Acta obst et gynec scandinav 47 132, 1968

From the Department of Obstetrics and Gynaecology (Prof Stig Kullander)
Malmö General Hospital University of Lund Malmö Sweden

VARIATIONS IN THE MILK EJECTION REFLEX IN WOMEN DURING THE EARLY STAGES OF LACTATION

BY

LARS-ERIK SANDHOLM

The milk ejection or "let-down reflex in animals and in human beings has long been known

Ott and *Scott* (1910) showed that the milk ejection in lactating goats could be stimulated by an extract from the posterior pituitary

Gaines (1915) underlined the difference between the secretion and the ejection of milk. Later experiments with purified posterior pituitary extract have shown that milk ejection is elicited mainly by oxytocin which causes contraction of the myo-epithelial cells around the glandular alveoli in the breasts. This contraction expresses the milk into the lactiferous ducts and sinuses, whence it can be removed by suckling or milking (*Richardson* 1949 *Cross* 1961) The pressure in the mammary gland is dependent on the amount of milk in the breast and the contractions of the myo-epithelial cells

In previous investigations of milk ejection in women interest was confined to the appearance of the milk on the mamilla (*Beller et al* 1958 *Douglas et al* 1957 *Nickerson* 1954)

Cross and *Harris* (1952) devised a method for measuring the intramammary pressure in rabbits. They introduced a fine cannula or plastic catheter into one of the excretory ducts of the mamilla and recorded the intramammary pressure with a transducer and an amplifier The method permits the recording of responses to intravenous doses of oxytocin down to 0.5 mU The technique has been refined by injection of the test solution

milk available In multiparae but not in primiparae there was a correlation between the amount of milk removed by the child and the total amount available

The relationship between the milk ejection response and the amount of milk secreted after suckling stimulation has not been previously investigated in women. Primiparae are more prone to emotional disturbances in the early stages of lactation because it is a new experience for them. This may affect their response to suckling stimulation.

This paper is concerned with variations in the secretion of milk and in the milk ejection reflex during the early stages of lactation in primiparae and multiparae.

Material and Methods

The material consisted of 5 primiparae and 5 multiparae (one Para III and four Para II) The women had been delivered at term and without complications. During the study period the puerperium was uncomplicated and the children increased in weight at a normal rate.

Examination technique Recordings were made at 10 a.m. on the 2nd 4th 6th, 8th and 10th days after delivery The women left the hospital in the afternoon of the 6th day and returned for study on the 8th and 10th days.

The pressure in one of the breasts was measured with Friedman's technique The breast had been emptied four hours previously

A perspex cup was placed over one of the mamillae and was connected by a tube to the transducer (Elema EMT 30) The system was filled with physiological saline The recordings were amplified using an electromanometer (Elema) and permanent tracings made on a recorder (Varian G 10)

The responsiveness to oxytocin was first tested by giving the women a dose of 10 mU Syntocinon® into a cubital vein. When the intramammary pressure had returned to its original level the child was placed to the contra-lateral breast and was allowed to suck for as long as it wished—usually about 10 minutes.

Chan (1965) found that α and β-adrenergic blockade of the vascular responses to adrenalin did not abolish the inhibitory effect on milk ejection. These findings indicate that the milk ejection inhibitory effect of adrenalin is an intrinsic activity of the hormone acting directly on the mammary tissue and is not the result of its vasoconstrictor activity preventing oxytocin from reaching the mammary gland.

Adrenalin can also induce contraction of the muscle cells forming a sphincter around the smallest lactiferous ducts in the breasts (cf *Zaks* 1962)

In infusion experiments on rabbits *Kullander* (1963) found that an increase in the concentration of vasopressin relative to that of oxytocin in the blood results in a fall of the intramammary pressure

The relation between milk ejection and secretion is obscure. The suckling or milking stimulus induces release of the pituitary hormones which are essential for the maintenance of lactation and milk removal Suckling results in a rapid discharge of prolactin, ACTH and oxytocin (*Meites et al.* 1963)

Several workers have postulated the existence of a humoral link between the release of both posterior and anterior hypophyseal hormones after suckling stimulation They have suggested that oxytocin is responsible for the release of prolactin and possibly other galactopoetic hormones (*Benson* and *Folley* 1956 1957)

Later experimental evidence is against the view that oxytocin induces prolactin release Neither oxytocin nor vasopressin stimulates prolacin secretion from pituitary tissure *in vitro* (*Meites et al* 1963)

It is conceivable that a strong milk ejection reflex might result in a better removal of milk and thereby in a stronger stimulation of its secretion If the milk ejection reflex is inhibited the breast will not be properly emptied with a consequent fall in the amount of milk removed.

Kullander (1960) studied the effect of oxytocin on the tension of the breasts and on normal lactation in the early puerperium On the fourth and fifth days after parturition the amount of milk removed by the child was compared with the total amount of



Fig. 2. Pressure recording during breast feeding. 1 denotes spontaneous contraction. At 2 the patient received 10 mU of Syntocinon® intravenously. Between 3 and 4 the child was put to the other breast. The areas of the pressure curves after injection of oxytocin and feeding were measured planimetrically

tocin. In several of the multiparae the pressure began to rise while they were preparing themselves to feed the child, *i.e.* before the child was put to the breast and before the injection of oxytocin. When questioned, these women said that they had thought about the child or about feeding it.

Some 20 to 60 sec. after the child had been put to the breast the IMP rose rapidly to reach a level at least as high as that recorded after injection of 10 mU of Syntocinon® after which the recording showed a plateau with small peaks. Towards the end of the feed the contractions were weaker. During the recording the increases in pressure often occurred after the child had sucked strongly.

Fig. 3 shows the amounts of milk removed by the child and the pump, respectively, as well as the milk ejection values. Corresponding values are given in Tables I and II. The milk ejection values after injection of oxytocin are given in Table III.

In all of the women, except No. 7, the amounts of milk and the milk ejection values increased after the 2nd day.

The amounts of milk were larger in multiparae than in primiparae only on the 2nd day.

The milk ejection values were higher in the multiparae than in the primiparae except in No. 5.

With the exception of No. 7 the women with the largest amounts of residual milk also showed the highest milk ejection values.



Fig 1 Recording of the intramammary pressure according to Friedman.

In order to estimate the strength of the milk ejection reflex at each examination the area between the base line and the pressure recording was measured planimetrically for the entire feed. This method gives a more exact evaluation of milk ejection activity than if only the maximum pressure peaks are analyzed.

The conditions under which the measurements were made were kept as uniform as possible

The amount of milk removed was calculated from the difference in weight of the child before and after it had been put to the breast Immediately after the child had been fed the milk still in the breast was removed by an electric pump and weighed.

Results

A typical pressure recording is shown in Fig 2 which illustrates how the milk ejection value was determined planimetrically The pressure began to rise 15–20 sec. after the injection of the oxy



Fig. 2. Pressure recording during breast feeding. 1 denotes a spontaneous contraction. At 2 the patient received 10 mU of Syntocinon® intravenously. Between 3 and 4 the child was put to the other breast. The areas of the pressure curves after injection of oxytocin and feeding were measured planimetrically.

tocin. In several of the multiparae the pressure began to rise while they were preparing themselves to feed the child, i.e. before the child was put to the breast and before the injection of oxytocin. When questioned, these women said that they had thought about the child or about feeding it.

Some 20 to 60 sec. after the child had been put to the breast the IMP rose rapidly to reach a level at least as high as that recorded after injection of 10 mU of Syntocinon®, after which the recording showed a plateau with small peaks. Towards the end of the feed the contractions were weaker. During the recording the increases in pressure often occurred after the child had sucked strongly.

Fig. 3 shows the amounts of milk removed by the child and the pump respectively as well as the milk ejection values. Corresponding values are given in Tables I and II. The milk ejection values after injection of oxytocin are given in Table III.

In all of the women except No. 7 the amounts of milk and the milk ejection values increased after the 2nd day.

The amounts of milk were larger in multiparae than in primiparae only on the 2nd day.

The milk ejection values were higher in the multiparae than in the primiparae except in No. 5.

With the exception of No. 7 the women with the largest amounts of residual milk also showed the highest milk ejection values.

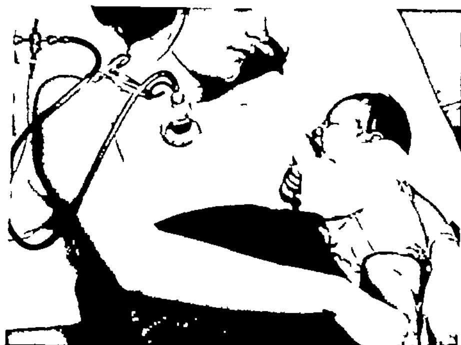


Fig. 1 Recording of the intramammary pressure according to Friedman.

In order to estimate the strength of the milk ejection reflex at each examination the area between the base line and the pressure recording was measured planimetrically for the entire feed. This method gives a more exact evaluation of milk ejection activity than if only the maximum pressure peaks are analyzed.

The conditions under which the measurements were made were kept as uniform as possible

The amount of milk removed was calculated from the difference in weight of the child before and after it had been put to the breast. Immediately after the child had been fed, the milk still in the breast was removed by an electric pump and weighed.

Results

A typical pressure recording is shown in Fig 2 which illustrates how the milk ejection value was determined planimetrically The pressure began to rise 15–20 sec. after the injection of the oxy

Table I *Milk Removed from Each Women on Each Occasion* (Gm)
C amount removed by the child. P = amount removed by the pump

Primiparae

Day	Case 1		Case 2		Case 3		Case 4		Case 5	
	C	P	C	P	C	P	C	P	C	P
2	40	10	20	0	30	0	10	0	30	0
4	90	85	60	60	90	10	60	5	70	130
6	100	40	100	10	80	90	70	0	20	200
8	100	15	90	20	80	10	60	0	140	40
10	100	15	90	40	110	50	100	0	100	30

Multiparae

Day	Case 6		Case 7		Case 8		Case 9		Case 10	
	C	P	C	P	C	P	C	P	C	P
2	40	20	0	50	20	10	20	10	20	40
4	70	40	40	10	40	20	40	20	80	100
6	50	120	60	10	90	10	90	10	80	120
8	60	80	60	10	60	10	60	10	90	30
10	80	20			90	10	90	10	100	70

Discussion

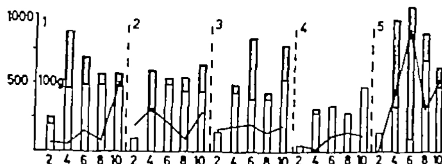
In an investigation of this kind there are several factors which cannot be estimated or eliminated.

Newton and *Newton* (1948) showed how maternal stress can inhibit the ejection of milk.

The tolerance to stress varies from one individual to another so that a given series can never be uniform in this respect. All the patients in the present investigation were quiet and coopera tive

The intramammary pressure varies considerably with the amount of milk in the breast.

The strength with which the child sucks depends, among other things, on its general development and its level of wakefulness.



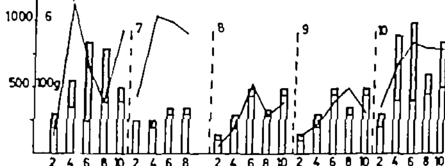


Fig 3 Amounts of milk and the individual milk ejection values. Blank columns denote the amount of milk removed by the child. Hatched columns denote the amount of milk pumped off Milk ejection values as measured by the planimeter see Fig. 2 and text

As a rule the injection of Syntocinon® also produced a larger increase of the mammary pressure in the multiparae than in the primiparae

The spontaneous rises in pressure sometimes occurring before the child was put to the breast, were just as large as those produced by the intravenous injections of 10 mU of Syntocinon®

A child rarely removes more than 100 g of milk at a single feed, even when the mother has still more in the breast.

In most of the women the milk ejection values and sometimes also the amounts of milk decreased on the 8th day

Table III. Increase in Mammary Pressure After Intravenous Injection of 10 mU of Syntocinon®

Primiparae					
Day	Case 1	Case 2	Case 3	Case 4	Case 5
2	16	–	4	7	–
4	14			12	32
6				21	6
8	39		32	24	–
10	20	17		146	71

Multiparae					
Day	Case 6	Case 7	Case 8	Case 9	Case 10
2	9		8	10	91
4		275	10	59	145
6	113	174	78	60	319
8	80	163			190
10	102		119	171	240

milk ejection values were often high. Since the pressures were recorded in a breast from which the child had been fed previously it is not possible to draw any conclusions about the relation between the amount of milk and milk ejection. The pressure is presumably dependent on the distension of the gland with secretion In an empty breast, contraction of the myo-epithelial cells will have but little effect because the pressure losses in the elastic tissue on the way to the measuring point are large If however the lactiferous ducts are distended by milk the pressure losses will be smaller The increase in the milk ejection values after the second day probably depend on an increased secretion of milk and better filling of the breast. The high pressure in women with large amounts of *residual milk* might, perhaps be due to their having a more abundant secretion of milk in the breast where the measurements were made.

One patient (No 7) differed from the others. She had high ejection values and the amounts of milk removed by suckling and

Table II. *Milk Ejection Values During Breast Feeding* (Cf. Figs. 2 and 3) (as measured by the planimeter)

Primiparae

Day	Case 1	Case 2	Case 3	Case 4	Case 5
2	62	210	170	55	0
4	54	325	186	25	455
6	158	209	216	123	914
8	73	88	152	210	330
10	506	291	209	117	560

Multiparae

Day	Case 6	Case 7	Case 8	Case 9	Case 10
2	134	431	42	132	350
4	1204	1071	189	220	678
6	652	1003	553	378	850
8	401	922	295	492	792
10	934		409	316	787

All of the children in the present investigation grew normally during the period of study In an attempt to keep the test condi tions as uniform as possible with regard to the degree of filling of the breast and level of wakefulness of the child the measurements were always made in the same breast and at the same hour of the day

The amount of milk removed by the child and by the pump could be measured with satisfactory accuracy

The pressure was recorded with Friedman s relatively crude method. The cannulation technique is about 10 times more sensi tive but it causes considerable inconvenience to the patient. Measurements with Friedman s technique could be made repeatedly without the examination causing any emotional stress

In several of the patients good agreement was found between the variation in the amounts of milk and the milk ejection values In these cases the amounts of residual milk were small However in the patients with relatively large amounts of residual milk the

smaller than previously This agrees with the well known fact that the secretion of milk tends to be less during the first few days after the mothers have returned home from the maternity department.

SUMMARY

In 5 multiparae and in 5 primiparae the amount of milk and the ejection of milk during lactation was studied from the 2nd to the 10th day after parturition. No significant difference in milk secretion was found between the groups. The milk ejection response during suckling and the increase in the intramammary pressure after intravenous injection of oxytocin was much higher in the multiparae.

In order to explain this difference it is suggested that lactation may impose a greater stress on primiparae and this may inhibit the peripheral effects of oxytocin.

REFERENCES

Beller F K Krumholz K H and *Zeitlinger K.*, Acta Endocrin. 29 1 1958
Benson, G K., and *Folley S. J* Nature (London) *177* 700 1956
J Endocrin. *14* 1 1957
Ibidem *16* 189 1957
Berde B and *Cerletti A.* Gynaecologia *144* 275 1957
Chan W Y J Pharmacol *147* 48 1965
Chaudhury R R Chaudhury M R., and *Lu F C.*, Brit. J Pharmacol *17* 305 1961
Cobo E Gaitan E and *Mizrachi M* Amer J Obstet. Gynec. *91* 905 1965
Cowie A T in *Kon S K* and *Cowie A T* ed. Milk: the mammary gland and its secretion, vol 1 Academic Press London 1961
Cross B A and *Harris G W J* J Endocrin. *8* 148, 1952
Cross B A and *van Dyke H B* J Endocrin 9 232, 1953
Cross B A J Endocrin *12* 15 1955
Ibidem *12* 29 1955
in Caldeyro-Barcia R and Heller H ed Oxytocin, Pergamon Press, London 1961
Denamur R Dairy Sci Abstr 27 (5) 193, 1965
Ibidem 27 (6) 263 1965
*Dougl*a*s R G Kramer E E* and *Bonsnes R W* Amer J Obstet. Gynec. *72* 1206, 1957
Friedman E A Amer J Obstet Gynec. 80 119 1960

the pump were small The child was put to the same breast every time If the breasts differed considerably from one another in their functional capacity and if the recordings had been regularly made on the better side and the child had been fed on the contra lateral side it would be possible to explain this remarkable result. The child increased in weight at a normal rate without supplementary food.

No significant difference was found between the multiparae and the primiparae with regard to the amounts of milk measured. In multiparae lactation is established earlier than in primiparae which results in the differences observed on the 2nd day

The milk ejection values and the responses to oxytocin given intravenously were higher in the multiparae than in the primiparae Since the two groups did not differ from one another with regard to the amount of milk, the difference in pressure cannot be explained by a better filling of the breasts of multiparae

The most likely explanation is that the peripheral sensitivity to oxytocin is lower in primiparae Central inhibition of the milk ejection reflex is a less likely explanation because in this case intravenous oxytocin would have had the same effect in both groups

Peripheral inhibition of the milk ejection reflex by emotional stress might be transmitted by adrenalin or possibly vasopressin (*Kullander* 1963)

One might imagine that breast feeding is more liable to cause psychological stress in primiparae in whom it is a new experience than in multiparae

Kullander (1960) has shown that there is better agreement in multiparae between the amount of milk removed by the child and the total amount of milk.

The tendency of the milk ejection values to decrease which was noted on the 8th day in both the multiparae and the primiparae may also have been due to some extent, to stress. The patients were in hospital until after measurement on the 6th day during which time they were free from all housework and care of the child. On the 8th and 10th days they came with their children to the hospital for study

The amount of milk measured on the 8th day was sometimes

Sandholm, Lars-Erik, Acta obst. et gynec. scandinav. 47 143, 1968
From the Department of Obstetrics and Gynaecology (Prof. Stig Kullander)
Malmö General Hospital, University of Lund, Malmö, Sweden

THE EFFECT OF INTRAVENOUS AND INTRANASAL OXYTOCIN ON INTRAMAMMARY PRESSURE DURING EARLY LACTATION

BY

LARS-ERIK SANDHOLM

Oxytocin has been used for more than 40 years for inducing and stimulating uterine contractions. The hormone is broken down in the gastro-intestinal tract and is therefore given parenterally, sublingually or intranasally.

Intravenous infusion is effective and safe. The effect of the hormone given by this route has been studied extensively. The relationships between dose and response have been studied by *Theobald et al.* (1948), *Engström* (1959), *Poseiro* and *Noriega Guerra* (1961).

When administered sublingually the hormone must be given in a dose of 200–600 IU to produce an effect (*Donaldson* 1920, *Hamill* 1920, *Knaus* 1926, *Dillon et al.* 1960, *Rice* and *Benson* 1961, *Masheter* 1965).

The intranasal route was tried mainly in the twenties and thirties and the effect was found to be uncertain and irregular (*Hofbauer et al.* 1927, *Slemons* 1932, *Morton* 1933, *Holmes* 1934). *Borglin* (1961, 1962) gave oxytocin intranasally with a special polyethylene tube and reported good results. He used doses of 5 to 40 IU. Oxytocin sprayed intranasally was used by *Baumgarten* and *Hofhansl* (1961) as a test for labour and *Whitfield* (1965) for stimulating uterine contractions.

Oxytocin induces contractions not only of the myometrium, but also of the myo-epithelial cells within the breast (*Richardson* 1949, *Linzell* 1953). The contraction results in an increase in

Fitzpatrick R. J In. Caldeyro-Barcia R. and Heller H ed. Oxytocin, Pergamon Press London 1961

Gaines W L Amer J Physiol., *38* 285 1915

Gaitan E *Cobo E* and *Mizrachi M*., J Clin. Invest. *43* 12, 230 1964

Kullander S Gynaecologia *150* 213 1960

– Acta Endocrin. *44* 313 1963

Meites J *Nicoll C N* and *Talwalker P K* in. Nalbanov A. V ed. Advances in Neuroendocrinology University of Illinois Press Urbana 1963

Newton M and *Newton N R*. J Pediatrics, *33* 698 1948

Nickerson K, *Bonsnes R. W*., *Douglas R. G* *Condliffe P* and *Du Vigneaud V* Amer J Obstet Gynec. *67* 1028 1954

Ott I and *Scott J C* Proc. Soc. Exper Biol. Med. *8* 43 1910–11

Richardson K. C. Proc. Royal Soc. London *30* 136 1949

Sala N *Sica Blanco Y* and *Cobo E*. Tercer Congreso Uruguayo de Ginecotocologia *II* 297 1960

Sica Blanco Y *Sala N* *Gonzalez Panizza V H* and *Caldeyro-Barcia R*, Tercer Congreso Uruguayo de Ginecotocologia, *II* 282 1960

Zaks M G The Motor Apparatus of the Mammary Gland, Oliver and Boyd Ltd. London 1962

Received on April 24 1967

were examined on the fourth day after an uncomplicated delivery. None had received any premedication. The IMP was measured by the method of *Sica Blanco et al.* (1959). The breast was washed with an antiseptic solution and one of the ducts was dilated with a lacrimal duct dilator. A plastic catheter (Portex PP 50) was introduced about 2 cm into the duct. The catheter was filled with saline and connected to a transducer (Elema, EMT 30). A continuous pressure tracing was made with a recorder (ABEM Ultralette). Oxytocin was given into an antecubital vein as single injections or continuously with an infusion pump (Braun, Melsungen). The oxytocin preparation used was Partocon® AB Ferring, Malmö, Sweden. For intranasal use it contains 100 IU oxytocin/ml. All oxytocin used was from the same batch.

Three patients were given oxytocin intranasally in doses of 2.5, 1.25 and 0.625 IU in order to find the smallest dose which produced an increase in the IMP. In these cases the oxytocin solution was diluted with saline so that the volume of fluid given was always 0.05 ml.

In the other 15 patients the IMP was first measured for some minutes, after which oxytocin was infused intravenously at a rate of 5 mU/min. When the pressure level had become stable—after about 10 minutes—the rate of infusion was increased to 10, 20 or 50 mU/min. When the pressure had stabilised again the infusion was stopped until the IMP had returned to the original level. Then, oxytocin was given intranasally and the IMP was recorded as long as any increase was demonstrable. The patients were divided into three equal groups which received either 5 IU (0.05 ml), 10 IU (0.1 ml) or 20 IU (0.2 ml) oxytocin respectively.

The effect of each dose, given by infusion or intranasally, was judged on the basis of a 5-minute segment of the record. The area between the base-line and the IMP-curve was measured with a planimeter. The values obtained were used to compare different parts of the record. After the single intravenous injections of oxytocin the entire area showing the effect was determined in the same way for each dose and the values obtained were used to calculate the dose-response relationship.

intramammary pressure (IMP) which is followed by milk ejection"

The IMP in women can be recorded by the method of *Sica-Blanco et al* (1959) A thin plastic catheter is introduced into one of the mammary ducts and connected to a transducer and recorder

The myo-epithelial cells are more sensitive than the myometrium to a single intravenous injection of oxytocin (*Caldeyro-Barcia et al* 1961) The sensitivity increases with advancing pregnancy and is greatest in the puerperium.

Friedman (1960) and *Friedman* and *Sachtleben* (1961) who used a less sensitive technique studied the IMP in puerperal women after oxytocin given sublingually or with a nasal spray The linguettes began to act after 6 minutes and the effect persisted for 30 minutes The spray rapidly produced an effect of short duration *Wiederman* and *Stone* (1962) who used Sica-Blanco s technique studied the effect of oxytocin given as an intranasal spray and found that the smallest dose capable of producing an effect on the IMP was 2 IU The IMP rose after 1 minute and the increase in pressure persisted for 10–15 minutes.

Following *Borglin s* report (1961) intranasal oxytocin administration was used in this hospital as a routine method for the stimulation and induction of uterine contractions. The intranasal doses of oxytocin required are relatively large compared with those needed intravenously

It is not known how much of the intranasal oxytocin reaches the blood stream and if there is a greater risk of over dosage than if the hormone is given intravenously?

This paper is concerned with a comparison of the response of the IMP to oxytocin given intravenously as an infusion or by single injections with that following intranasal oxytocin introduced by means of a polyethylene tube as described by *Borglin* (1961)

Clinical material and methods

The clinical material consisted of 18 healthy mothers (14 multiparae and 4 primiparae) who were nursing their children All

were examined on the fourth day after an uncomplicated delivery. None had received any premedication. The IMP was measured by the method of *Sica Blanco et al.* (1959). The breast was washed with an antiseptic solution and one of the ducts was dilated with a lacrimal duct dilator. A plastic catheter (Portex PP 50) was introduced about 2 cm into the duct. The catheter was filled with saline and connected to a transducer (Elema, EMT 30). A continuous pressure tracing was made with a recorder (ABEM, Ultralette). Oxytocin was given into an antecubital vein as single injections or continuously with an infusion pump (Braun Melsungen). The oxytocin preparation used was Partocon® AB Ferring, Malmö, Sweden. For intranasal use it contains 100 IU oxytocin/ml. All oxytocin used was from the same batch.

Three patients were given oxytocin intranasally in doses of 2.5, 1.25 and 0.625 IU in order to find the smallest dose which produced an increase in the IMP. In these cases the oxytocin solution was diluted with saline so that the volume of fluid given was always 0.05 ml.

In the other 15 patients the IMP was first measured for some minutes, after which oxytocin was infused intravenously at a rate of 5 mU/min. When the pressure level had become stable—after about 10 minutes—the rate of infusion was increased to 10, 20 or 50 mU/min. When the pressure had stabilised again the infusion was stopped until the IMP had returned to the original level. Then, oxytocin was given intranasally and the IMP was recorded as long as any increase was demonstrable. The patients were divided into three equal groups which received either 5 IU (0.05 ml), 10 IU (0.1 ml) or 20 IU (0.2 ml) oxytocin respectively.

The effect of each dose, given by infusion or intranasally, was judged on the basis of a 5-minute segment of the record. The area between the base-line and the IMP-curve was measured with a planimeter. The values obtained were used to compare different parts of the record. After the single intravenous injections of oxytocin the entire area showing the effect was determined in the same way for each dose, and the values obtained were used to calculate the dose-response relationship.

intramammary pressure (IMP) which is followed by milk ejec tion

The IMP in women can be recorded by the method of *Sica Blanco et al* (1959) A thin plastic catheter is introduced into one of the mammary ducts and connected to a transducer and recorder

The myo-epithelial cells are more sensitive than the myometrium to a single intravenous injection of oxytocin (*Caldeyro-Barcia et al* 1961) The sensitivity increases with advancing pregnancy and is greatest in the puerperium.

Friedman (1960) and *Friedman* and *Sachtleben* (1961) who used a less sensitive technique studied the IMP in puerperal women after oxytocin given sublingually or with a nasal spray The linguettes began to act after 6 minutes and the effect persisted for 30 minutes The spray rapidly produced an effect of short duration *Wiederman* and *Stone* (1962) who used Sica Blanco s technique studied the effect of oxytocin given as an intranasal spray and found that the smallest dose capable of producing an effect on the IMP was 2 IU The IMP rose after 1 minute and the increase in pressure persisted for 10–15 minutes.

Following *Borglin s* report (1961) intranasal oxytocin administration was used in this hospital as a routine method for the stimulation and induction of uterine contractions The intranasal doses of oxytocin required are relatively large compared with those needed intravenously

It is not known how much of the intranasal oxytocin reaches the blood stream and if there is a greater risk of over dosage than if the hormone is given intravenously?

This paper is concerned with a comparison of the response of the IMP to oxytocin given intravenously as an infusion or by single injections with that following intranasal oxytocin introduced by means of a polyethylene tube as described by *Borglin* (1961)

Clinical material and methods

The clinical material consisted of 18 healthy mothers (14 multiparae and 4 primiparae) who were nursing their children All



Fig. 3 Patient 4 Paper speed 1 minute between markings.—1 Start of infusion of oxytocin 5 mU/min.—2. Increase of rate of infusion to 20 mU/min. —3 End of infusion —4 Oxytocin intranasally 10 IU

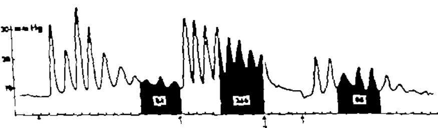


Fig. 4 Patient 7 Paper speed 1 minute between markings —1 Infusion of oxytocin 5 mU/min —2. Increase of rate of infusion to 10 mU/min.—3. End of infusion —4 Oxytocin intranasally 5 IU

Results

The smallest intranasal dose of oxytocin which produced an increase of the IMP was 1.25 IU in two cases and 2.5 IU in another (Fig 1)

Oxytocin given as single intravenous injections gave a dose-response curve showing a linear relationship between the pressure and the logarithm of the dose.

An incidental finding in patient 20 (Fig. 1) was a spontaneous contraction of the same intensity as that produced by a single intravenous injection of 10 mU oxytocin. The end of this recording shows the pattern of contractions seen when the contralateral breast is emptied by an electric breast pump (AB Einar



Fig 1 Patient 20. Paper speed 1 minute between markings.—1 Oxytocin intranasally 2.5 *IU*.—2. Oxytocin intravenously 10 mU.—3. Spontaneous contraction.—4 Oxytocin intravenously 5 mU.—5. Oxytocin intravenously 2 mU.—6. Oxytocin intranasally 1.25 IU.—7 Electric breast pump on contralateral breast. The dark segments of the curve were measured with a planimeter. The figure for each area is given in the white square.

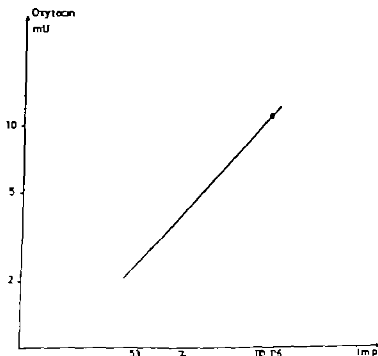


Fig 2. Same experiment as Fig 1.—IMP values after intravenous injections of oxytocin are plotted against the logarithms of the oxytocin dose. The circle denotes the IMP-value of a spontaneous contraction.



Fig 3 Patient 4 Paper speed 1 minute between markings —1 Start of infusion of oxytocin 5 mU/min —2 Increase of rate of infusion to 20 mU/min. —3 End of infusion —4 Oxytocin intranasally 10 IU

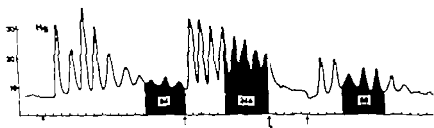


Fig 4 Patient 7 Paper speed 1 minute between markings.—1 Infusion of oxytocin 5 mU/min —2 Increase of rat of infusion to 10 mU/min —3. End of infusion —4 Oxytocin intranasally 5 IU

Results

The smallest intranasal dose of oxytocin which produced an increase of the IMP was 1.25 IU in two cases and 2.5 IU in another (Fig. 1)

Oxytocin given as single intravenous injections gave a dose-response curve showing a linear relationship between the pressure and the logarithm of the dose

An incidental finding in patient 20 (Fig. 1) was a spontaneous contraction of the same intensity as that produced by a single intravenous injection of 10 mU oxytocin The end of this recording shows the pattern of contractions seen when the contra lateral breast is emptied by an electric breast pump (AB Elmar

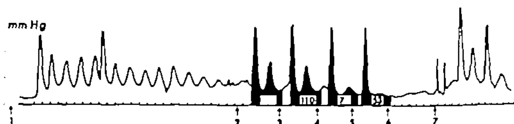


Fig. 1 Patient 20 Paper speed 1 minute between markings.—1 Oxytocin intranasally 2.5 IU —2. Oxytocin intravenously 10 mU —3. Spontaneous contraction.—4 Oxytocin intravenously 5 mU —5 Oxytocin intravenously 2 mU —6. Oxytocin intranasally 1.25 IU —7 Electric breast pump on contralateral breast. The dark segments of the curve were measured with a planimeter The figure for each area is given in the white square

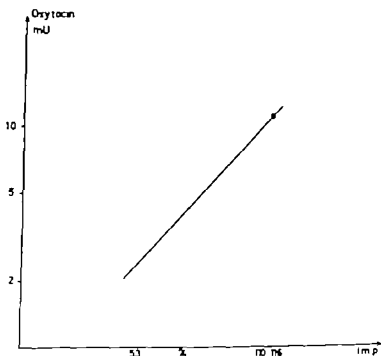


Fig. 2. Same experiment as Fig. 1 —IMP values after intravenous injections of oxytocin are plotted against the logarithms of the oxytoci dose The circle denotes the IMP value of a spontaneous contraction.

duce an effect on the IMP was about 2 IU This is in agreement with the results obtained by *Wiederman* and *Stone* (1962) who used an oxytocin spray After a single intravenous injection the IMP-curve showed a single or when the dose was larger 2–3 decreasing contractions. An intranasal dose on the other hand, produced repeated contractions for 10–20 minutes. The curve was not different from that obtained after an intravenous infusion. Presumably the rate of absorption from the nasal mucosa is slow and fairly constant.

Suckling at one of the nipples produces a pressure curve from the contralateral breast which resembles that seen after the intra venous infusion of oxytocin in a dose of 4–5 mU/min. (*Sica Blanco et al.* 1960) Fig 1 shows that suction with an electric breast pump of suitable construction produces a similar IMP–curve

Since the effect of oxytocin given intranasally resembles that of intravenous infusion it seemed interesting to see whether larger increases in IMP could be produced by larger doses of oxytocin given intranasally and intravenously and whether any dose-response relationship could be demonstrated. The area under the IMP curve as measured with a planimeter for a 5-minute period may be regarded as representing the work performed by the myo-epithelial cells. The beginning of the infusion is soon followed by a few strong contractions at fairly long intervals. The basal pres sure level in the breast becomes higher and the peaks become lower but more frequent. When the rate of infusion is increased, the curve again shows some high peaks and then smoothes out, often at a somewhat higher level than before (Fig. 4)

The latter part of the curve probably indicates that the blood concentration of oxytocin has become steady The more even part of the curve should, therefore be used in comparing the effects of different infusion doses. The results of the investigation would perhaps have been more reliable if the infusions had been con tinued for more than 10–15 minutes. On the other hand, the effect of an intranasal dose did not last for a longer period. Furthermore more prolonged infusion might result in fatigue of the myo-epithelial cells and consequently with weaker contrac tions.

ble I *IMP values During 5 Minute Periods after Different Doses of Oxytocin Given by ravenous Infusion and Intranasally*

Case	8	5	7	11	12	3	4	15	16	17	6	9	10	13
5	112	306	84	155	82	293	157	138	91	124	213	15?	207	245
10						299	144				264			
20	109	233	246						89			143		
50				176	163			138		117			224	302
5 U	102	304	88	152	107									
10 U						347	112	102	145	87				
20 U											197	99	211	306

Table II *Duration of Effect of Different Doses of Oxytocin Given Intranasally*

5 U	8	9	11	13	11										
0 U						20	19	18	15	15					
0 U											25	18	20	23	15

Egnell Trollhättan Sweden) The results of the intravenous in fusion of different doses of oxytocin and subsequently of intra nasal oxytocin are given in Tables I and II

Fig 3 (patient 4) and Fig 4 (patient 7) show typical curves and illustrate the method of measuring the area under the curve

After the intranasal administration of oxytocin the pressure showed an increase within 1–1.5 minutes which persisted for 10–20 minutes

Discussion

Several workers have shown that a single small intravenous dose of oxytocin increases the IMP the increase varying logarithmically with the size of the dose (*Wiederman and Stone* 1962) This was also found in the present investigation (Fig 2)

When given intranasally the dose of oxytocin necessary to pro-

SUMMARY

Recordings were made of the intramammary pressure (IMP) in lactating women on the fourth day post partum. Oxytocin was given intravenously as single injections as an intravenous infusion, or intranasally. The response of the IMP was studied. In one case the IMP was recorded during suction stimulation of the other breast with an electric breast pump.

Results

1. The response of the IMP to small intravenous doses of oxytocin gave a dose response curve which showed a linear relationship between pressure and the logarithm of the dose.
2. Intravenous infusions of oxytocin in increasing doses did not produce the same distinct response.
3. The smallest intranasal dose of oxytocin capable of producing an effect on the IMP was 1–2 IU
4. After intranasal administration the IMP-curve resembled that during an intravenous infusion or following suction stimulation of the contralateral nipple.
5. The effect of 5 IU oxytocin given intranasally lasted for about 10 minutes, that of 10 and 20 IU for up to 20 minutes.
6. The intranasal dose of oxytocin was about 100 times larger than that needed to produce a comparable effect by intravenous infusion.

REFERENCES

Baumgarten K and *Hofhansl, W* Zbl. Gynäk. 83 154 1961

Borglin N E Svenska Läkartidn. 58 2168 1961

Acta obst et gynec scandinav 41 238 1962

Caldeyro-Barcia R and *Sereno J A* in. Oxytocin, R Caldeyro-Barcia and H Heller ed. Pergamon Press, London 1961

Dillon T F Douglas R G., du Vigneaud V and *Barber M L* Obst & Gynec 15 587 1960

Donaldson, M Proc. Roy Soc Med. 14 1920–21

Engström L Induction of labour around full term especially by means of synthetic oxytocin in intravenous drip. Efficacy risks and indications Acta obst et gynec. scandinav 38 Suppl. 3 1959

Friedman E A., Am J Obst. & Gynec. 80 118 1960

The results of the experiments are given in Tables I and II from which it is clear that an increase in the infusion dose did not always produce a further increase of the IMP In some cases the pressure level remained unchanged or was even lower (Fig. 3) In some cases where the increased infusion dose did not result in an increase in the IMP it is possible either that the lower preceding dose had produced a maximal contraction or that the myo-epithelium had become fatigued and reacted less strongly In patients 6 7 11 12 and 14 the IMP increased after a larger infusion dose The pressure recordings in these cases can be used with advantage to estimate the effect of oxytocin given intranasally

A dose of 5 IU given intranasally produced a response corresponding roughly to that of an intravenous infusion at a rate of 5 mU/min In the group given 10 IU intranasally there was no case with a marked increase of IMP with increasing infusion dose. But here too the responses to oxytocin given intranasally were of the same size as to infusion of 5 mU/min

Of the group that received 20 IU intranasally there were two cases in which the response was as strong or stronger than after infusion of 50 mU/min

More striking was the difference in the duration of the effect after 5 IU it lasted for 10 minutes and after 10 or 20 IU it lasted for periods of up to 20 minutes

The linear relationship between response and the logarithm of the dose following a single injection of oxytocin intravenously could not be demonstrated with oxytocin infusions. It is therefore difficult to draw conclusions about the dose-response relationship when the hormone is given intranasally It appears however that a dose of 5 IU would not produce a more marked increase in pressure than infusions of 5–10 mU/min A dose of 5 IU given intranasally produced an effect that lasted for 10 minutes and corresponded to that elicited by an intravenous infusion of oxytocin 5 mU/minute for 10 minutes Larger intranasal doses can produce stronger responses if the absorption is good. The biggest difference between 5 IU and larger doses was that the larger doses produced a longer effect.

SUMMARY

Recordings were made of the intramammary pressure (IMP) in lactating women on the fourth day post partum. Oxytocin was given intravenously as single injections as an intravenous infusion or intranasally The response of the IMP was studied. In one case the IMP was recorded during suction stimulation of the other breast with an electric breast pump.

Results

1 The response of the IMP to small intravenous doses of oxy tocin gave a dose response curve, which showed a linear relationship between pressure and the logarithm of the dose.
2. Intravenous infusions of oxytocin in increasing doses did not produce the same distinct response.
3 The smallest intranasal dose of oxytocin capable of producing an effect on the IMP was 1–2 IU
4 After intranasal administration the IMP-curve resembled that during an intravenous infusion or following suction stimulation of the contralateral nipple.
5 The effect of 5 IU oxytocin given intranasally lasted for about 10 minutes that of 10 and 20 IU for up to 20 minutes.
6 The intranasal dose of oxytocin was about 100 times larger than that needed to produce a comparable effect by intra venous infusion.

REFERENCES

Baumgarten, K and *Hofhand W* Zbl Gynäk. 83 154 1961
Borglin N E Svenska Läkartidn 58, 2168 1961
Acta obst et gynec scandinav 41 238 1962
Caldeyro-Barcia, R and *Sereno J A* in Oxytocin R. Caldeyro-Barcia and H Heller ed Pergamon Press, London 1961
Dillon T F Douglas R G du Vigneaud, V and *Barber M L*, Obst. & Gynec 15 587 1960
Donaldson, M Proc Roy Soc Med. 14 1920–21
Fagerström L Induction of labour around full term especially by means of synthetic oxytocin in intravenous drip Efficacy risks and indications. Acta obst et gynec scandinav 38 Suppl 3 1959
Friedman E A Am J Obst & Gynec. 80 119 1960

Friedman E. A., and *Sachtleben* M R., Am. J Obst. & Gynec. 82, 846, 1961
Hamill P Proc. Roy Soc. Med. *14* 1920–21
Hofbauer J *Hoerner* J K. and *Oliver* K. S Am. J Obst. & Gyn. *14* 137 1927
Holmes O M., California Med. *41* 241 1934
Knaus H H Brit. Med. J *1* 234 1926
Lin ell J L. J Physiol. *130* 257 1955
Masheter H C. in. Advances in oxytocin research. J H. M. Pinkerton ed. Pergamon Press London 1965
Morton D G Am. J Obst. & Gynec. *26* 323 1933
Poseiro J J and *Noriega-Guerra* L. H., in. Oxytocin R. Caldeyro-Barcia and H. Heller ed. Pergamon Press London 1961
Rice R. D and *Benson* R. C., Obst. & Gynec. *17* 297 1961
Richardson K. C. Proc. Roy Soc. London *136* 30 1949
Sica Blanco Y *Mendez-Bauer* C. J *Sala* N., *Cabot* H M. and *Caldeyro-Barcia* R. Arch. Ginecol. Obst. Montevideo *17* 63 1959
Sica-Blanco Y *Sala* N *Gonzalez-Panizza* V H and *Caldeyro-Barcia* R., Tercer Congreso Uruguayo de Ginecotocologia *II* 283 1960
Slemons J M Am. J Obst. & Gyn. *23* 494 1932
Theobald G W *Graham* A. *Campbell* J *Gange* P D and *Driscoll* W J Brit med. J 2 123 1948
Whitfield C. R. in. Advances in oxytocin research, J H. M. Pinkerton ed. Pergamon Press London 1965
Wiederman J., and *Stone* M. C J Appl. Physiol. *17* 539 1962

Received on April 24 1967

Matheson, George W Davajan, Val, and Mishell, Daniel R Jr Acta obst. et gynec scandinav 47 [illegible], 1968

From the Department of Obstetrics and Gynecology Harbor General Hospital Torrance California and the University of California, Los Angeles, California U.S.A.

THE USE OF THE VACUUM EXTRACTOR A REAPPRAISAL

BY

GEORGE W MATHESON, VAL DAVAJAN, AND DANIEL R MISHELL JR.

Introduction

For many years obstetricians have attempted to develop an instrument that could be attached to the fetal scalp by suction in order to facilitate delivery of the infant. In 1954 and 1957 *Malmström* introduced a vacuum device that successfully met the specifications required for such an instrument. Since that time according to two recent reviews by *Malmström* (1964 and 1965) this vacuum extractor (V E.) has been extensively used throughout the world, in many places to the exclusion of the use of forceps for operative delivery

In spite of widespread European acceptance there have been infrequent reports of the use of the V E. in the United States. In the past two years, there has been only one report published describing the use of the V E. in a hospital in the U S. (*Bart* and *Newton* 1965) The paucity of data from this country in contrast to the widespread reports of its use elsewhere indicates that the vacuum extractor has not achieved acceptance for use in obstetrical practice in the United States

In 1962 *Kelly* and *Mishell* reported their experience with the acuum extractor in 202 deliveries at this Hospital. In their series as with other reports from U S institutions (*McCullough* and *Pisani* 1963 *Nyirjesy et al* 1963 *Pauly et al.*, 1963 *Tricomi*

N I H Postdoctoral Research Fellow University Hospital Uppsala, Sweden, 1967

et al 1961) the V E. was used electively in most instances in order to evaluate the instrument itself Since the earlier evaluation was performed, the V E. has continued to be used on this obstetrical service. However it has now been primarily used as an aid in the management of various obstetrical complications. It is the purpose of this report to review the use of the V E., not in terms of an elective clinical study of the instrument itself but as an integral part of the therapeutic armamentarium available to the obstetrician.

Materials and Methods

During the period from August 1 1963 to October 31 1965 there were 8847 deliveries on the obstetrical service. The Malmstrom vacuum extractor with hand pump was used in 168 of these deliveries for an incidence of 1.9 per cent. The V E. was used electively for training purposes with the vertex at the outlet in only 15 per cent of these 168 deliveries. In the remaining 85 per cent, 143 deliveries the use of the V E. was indicated because of an obstetrical complication Indications for use of the V E. in these 143 deliveries are listed in order of frequency (Table I)

Positional arrest in the second stage of labor accounted for 79 of the 143 indicated cases for an incidence of 55 per cent. Of these 79 46 were arrested in the occiput posterior position and 31 in the occiput transverse One fetus was a sinciput presentation, and one other was a brow presentation. *Prolonged second stage* in occiput anterior position accounted for 29 or 20 per cent, of the indicated V E. deliveries

Decreasing the duration of the second stage was the indication for use of the V E. in 20 instances or 14 per cent. Of these 20 patients 17 had pre-eclampsia in relatively poor control and the use of conduction anesthesia was contraindicated because of previous antihypertensive medication Bleeding secondary to abruptio placenta occurred in two of these patients. The remaining patient had a previous Cesarean section with subsequent vaginal deliveries

Fetal distress indicated use of the V E. in seven of the 143

AGA Corporation of America, South Plainfield, New Jersey

Table I. *Indications for Use of Vacuum Extractor in 143 Nonselective Cases (83 per cent of all vacuum extractor deliveries in this series)*

Indications		Number	Per cent
1 Positional arrests		79	55
A. Occiput posterior	46		
B Occiput transverse	31		
C. Sinciput	1		
D Brow	1		
2. Prolonged second stage		29	20
3 T decrease duration of second stage		20	14
A Pre-eclampsia	17		
B. Bleeding	2		
C Previous Cesarean section	1		
4 Fetal distress		7	5
5 Secondary uterine inertia		3	2
6 Inability to apply low forceps		2	1
7 Cervical dystocia		1	1
8 Prolapsed cord		1	1
9. Second twin		1	1
Total		143	100

cases. Four of these patients had the vertex unengaged at the time of application of the V E. *Secondary uterine inertia* unresponsive to oxytocin at 8 to 9 cm. dilatation led to three of the 143 extraction deliveries. *Inability to apply low forceps* satisfactorily was the primary indication to use the V E. in two instances. *Cervical dystocia* at 8 cm was an indication for use once. *Prolapsed cord* with an unengaged vertex was also an indication once. A *second twin* that failed to descend below—1 station even with oxytocic augmentation was the final indication. The station of the vertex at the time of V E. application for each of these indications is outlined in Table II

The mean parity of the total 168 patients was 1 4 prior to delivery Of the total, 85 or 51 per cent, were primigravidae Eighty percent of the patients were Caucasian and the remainder Negro. The average age was 23.5 years with 12 per cent over 35 years of age and 36 per cent under 20

Resident physicians performed 83 per cent of these deliveries

Table II *Station of the Fetal Vertex at the Time of Vacuum Extractor Application in 143 Indicated Deliveries*

Indication	Number of Cases			
	Unengaged	0 to +1	lower than +1	Total
Positional arrest		11	68	79
Prolonged second stage	1	9	19	29
Use to shorten second stage		6	14	20
Fetal distress	4	1	2	7
Uterine inertia	1	1	1	3
Inability to apply low forceps			2	2
Cervical dystocia		1		1
Prolapsed cord	1			1
Second twin	1			1
Total	8	29	106	143

and interns with resident supervision performed the remainder Conduction anesthesia was utilized in 35 per cent of the patients. The remainder had pudendal block or local anesthesia with or without intermittent N_2O-O_2 In some patients the relative con traindication to the use of general or conduction anesthesia was a major consideration although not the primary indication in choosing the V E. to aid delivery

The average weight of the infants delivered in this series was 7 lb 11 oz Five per cent of the newborns were premature (less than 2500 gm) and 17 per cent were of excessive weight (over 4000 gm.) Fifty-seven per cent of the infants delivered with the V E. were unfortunately not seen after discharge Of those in-fants who had complications causally related to the use of the V E. 60 per cent were seen in subsequent follow-up visits.

Results

The V E. succeeded in effecting a delivery in 89 per cent of the 168 cases. TheV E. failed in 19 cases or 11 per cent.

The V E. was applied successfully in nine cases after forceps

Table III. *Complications Related to Vacuum Extraction in 168 Deliveries*

	Number	Per cent
Maternal		
Cervical lacerations	11	7
Vaginal lacerations	9	5
Fetal		
Some degree of depression at birth	16	9.5
Scalp abrasions	13	8
Cephalohematoma	4	2
Overal perinatal mortality	7	4.2
Corrected perinatal mortality	3	1.8

failed to effect delivery In five of these nine cases, low forceps were abandoned because of inadequate anesthesia or inability to apply the forceps correctly In one of the remaining four patients Kielland forceps could not be satisfactorily applied to a vertex in ROT position at a +3 station. In two cases the Kielland rotation of an ROP presentation failed. Traction with Tucker McLaine forceps failed to deliver one infant with the vertex at a +1 station and a prolonged second stage. All these patients were delivered with the V E. without complication to mother or fetus.

In ten cases the V E. was applied more than once Four of these reapplications failed, necessitating Cesarean section. Three were delivered with forceps and only three were successfully delivered after reapplication of the V E.

No precise record was kept of the total length of time the V E. was applied to the vertex. It was possible to determine however that the V E. was attached to the fetal scalp for less than 30 minutes in 88 per cent of the cases and only in one instance was it attached for one hour

Of the 19 patients in whom the V E. failed to effect delivery four had a Cesarean section performed directly without an intervening trial of forceps. In the 15 remaining cases the vacuum extractor failures were followed by forceps. There were noted to be lacerations of the *fetal scalp* and/or the maternal cervix or

Table II. *Station of the Fetal Vertex at the Time of Vacuum Extractor Application in 143 Indicated Deliveries*

Indication	Number of Cases			
	Unengaged	0 to +1	lower than +1	Total
Positional arrest		11	68	79
Prolonged second stage	1	9	19	29
Use to shorten second stage		6	14	20
Fetal distress	4	1	2	7
Uterine inertia	1	1	1	3
Inability to apply low forceps			2	2
Cervical dystocia		1		1
Prolapsed cord	1			1
Second twin	1			1
Total	8	29	106	143

and interns with resident supervision performed the remainder Conduction anesthesia was utilized in 35 per cent of the patients. The remainder had pudendal block or local anesthesia with or without intermittent N_2O-O_2 In some patients the relative contraindication to the use of general or conduction anesthesia was a major consideration although not the primary indication, in choosing the V E. to aid delivery

The average weight of the infants delivered in this series was 7 lb 11 oz. Five per cent of the newborns were premature (less than 2500 gm) and 17 per cent were of excessive weight (over 4000 gm) Fifty seven per cent of the infants delivered with the V E. were unfortunately not seen after discharge Of those infants who had complications causally related to the use of the V E. 60 per cent were seen in subsequent follow up visits.

Results

The V E. succeeded in effecting a delivery in 89 per cent of the 168 cases TheV E. failed in 19 cases or 11 per cent.

The V E. was applied successfully in nine cases after forceps

delivered. The infant was initially depressed and had a large scalp hematoma with a small scalp laceration. The laceration became infected (the only scalp infection in the series) At 14 months of age no apparent abnormalities of the infant were noted.

There were no maternal deaths or serius maternal complications (Table III) In eleven cases, there were cervical lacerations all were repaired with ease In five of the eleven, the lacerations were probably unrelated to the vacuum extractor There were nine vaginal lacerations.

All the newborns had scalp ecchymosis and artificial caput. The caput (chignon) was markedly resolved twenty minutes following delivery and disappeared completely in every instance within 24 hours. The ecchymosis was barely visible in the vast majority of infants at the time of their discharge Only sixteen infants (9.5 per cent) of this high risk group had some degree of depression at birth. Thirteen infants (8 per cent) had scalp abrasions or lacerations. Only one infant electively delivered with the V E. had an abrasion and this healed rapidly without consequence. Only one infant, as noted above developed a scalp infection in the abrasion site There were four cephalohematomas which resolved without complication. Of these infants with complications of V E. delivery 60 per cent were seen at least once in follow-up clinic and no sequelae were noted.

The overall fetal mortality in this series was seven deaths or 4 2 per cent. Three deliveries were of stillborns who died prior to the onset of labor One infant died seven days after birth from congestive heart failure secondary to congenital heart disease. Therefore the corrected perinatal mortality was three deaths, or 1.8 per cent. The first of these occurred in an infant delivered by Cesarean section following an unsuccessful trial with both the V E. and forceps. Details of the case were presented above. The second was an 18 year old primigravida who was delivered with easy vacuum extraction The vertex had been at ROT position and + 1 station with a total second stage of two and one-half hours The membranes had ruptured 16 hours prior to delivery without evidence of amnionitis. The 7 lb 9 oz. male infant delivered without evidence of infection. There was a left occipit cephalohematoma and scalp abrasion The infant became febrile

vagina in six of these 15 cases where forceps were applied after a trial with the V E. had failed. All but one of these 15 were successfully delivered with the forceps. In eight cases the V E. had advanced the vertex from a higher station to the introitus but was accidently detached or deliberately removed and it was elected to complete delivery with forceps In three cases of positional arrest at a high station the vertex advanced but did not rotate After detachment of the V E., Kielland forceps were used for rotation and delivery In three of the 15 mechanical difficulty with the vacuum instrument itself required a change to forceps. The fifteenth case was a primigravida at term with a prolonged second stage and the vertex at a +1 station Traction with the V E. was attempted twice followed by a trial of forceps without success Cesarean section was then performed for cephalopelvic disproportion and a 10 lb 2 oz. male infant was delivered. The infant was initially depressed and expired at nine hours of age from atalectasis and aspiration pneumonia No gross evidence of CNS damage was noted at autopsy

In this series of 168 cases five (3 per cent) required Cesarean section One case was described above The second case was a brow presentation arrested at a +2 station in which a trial of V E. failed and a Cesarean section was performed. A third patient was a primigravida at term with an occipit posterior arrest at a +2 station After two applications of the V E. with intermittent traction for 30 minutes and no descent of the vertex the trial of V E. was abandoned and Cesarean section undertaken without complication The fourth Cesarean section followed prolapse of the umbilical cord with the vertex at a −1 station and the cervix 8 to 9 cm. dilated Vacuum extractor use was undertaken without success as preparations for Cesarean section were being made A 12 lb 1 oz. infant was delivered by Cesarean section without complication The final case was a 35 year old Caucasian female Gravida 2 Para 1 who had a positional arrest in the occiput transverse position at a −1 station. Three applications of the V E. were attempted with several pulls synchronous with contractions on each application The total time of attempted V E. delivery was 60 minutes After the third extractor failure a Cesarean section was undertaken and an 8 lb. 12 oz. male was

delivered. The infant was initially depressed and had a large scalp hematoma with a small scalp laceration. The laceration became infected (the only scalp infection in the series) At 14 months of age no apparent abnormalities of the infant were noted.

There were no maternal deaths or serius maternal complications (Table III) In eleven cases there were cervical lacerations all were repaired with ease. In five of the eleven, the lacerations were probably unrelated to the vacuum extractor There were nine vaginal lacerations.

All the newborns had scalp ecchymosis and artificial caput. The caput (chignon) was markedly resolved twenty minutes following delivery and disappeared completely in every instance within 24 hours. The ecchymosis was barely visible in the vast majority of infants at the time of their discharge Only sixteen infants (9.5 per cent) of this high risk group had some degree of depression at birth. Thirteen infants (8 per cent) had scalp abrasions or lacerations. Only one infant electively delivered with the V E. had an abrasion and this healed rapidly without consequence. Only one infant as noted above developed a scalp infection in the abrasion site There were four cephalohematomas which resolved without complication. Of these infants with complications of V E. delivery 60 per cent were seen at least once in follow-up clinic and no sequelae were noted.

The overall fetal mortality in this series was seven deaths or 4 2 per cent. Three deliveries were of stillborns who died prior to the onset of labor One infant died seven days after birth from congestive heart failure secondary to congenital heart disease. Therefore the corrected perinatal mortality was three deaths, or 1 8 per cent. The first of these occurred in an infant delivered by Cesarean section following an unsuccessful trial with both the V E. and forceps. Details of the case were presented above. The second was an 18 year old primigravida who was delivered with easy vacuum extraction The vertex had been at ROT position and +1 station with a total second stage of two and one-half hours The membranes had ruptured 16 hours prior to delivery without evidence of amnionitis. The 7 lb 9 oz. male infant delivered without evidence of infection. There was a left occipit cephalohematoma and scalp abrasion. The infant became febrile

at 36 hours and died at 72 hours of life of E. coli meningitis. The scalp abrasion was not infected. The third death occurred during delivery of an obese diabetic patient with pre-eclampsia. Fetal bradycardia was noted with the vertex at 0 station and complete cervical dilatation After nine pulls with the vacuum extractor the head was delivered. There was a 12 minute delay in delivery of the shoulders of a 10 lb 11 oz. female The infant took two or three agonal gasps but could not be resuscitated.

Discussion

The V E. was successfully used as an integral part of the obstetrical service It was used mainly for specific indications and not as an elective procedure This high incidence of indicated use of the V E. is in contrast to most other reports from U S institutions which have evaluated the instrument in large part on low risk elective cases. This has been a review of the experience of the *use* of the V E. as a working obstetrical tool not as a specific study to evaluate the instrument itself The V E. is not advocated to replace forceps but to act as a useful companion

In reviewing these 143 indicated cases it is apparent that in at least 30 patients (21 per cent) a Cesarean section or difficult traumatic forceps delivery was avoided by a relatively easy vacuum extraction Eleven of the 30 could only have been delivered by Cesarean section without the availability of the vacuum extractor In four of these cases forceps failed to effect the delivery Three cases of secondary uterine inertia unresponsive to oxytocin occurred prior to complete cervical dilatation There were four cases of marked sudden fetal distress with an incompletely dilated cervix or unengaged vertex that were expeditiously delivered by vacuum extractor One of these deliveries was accomplished without complication in 10 minutes after first diagnosing fetal distress at 7 cm dilatation and the vertex at −1 station

In the other 19 of these 31 cases delivery might have been effected vaginally but would have necessitated difficult forceps operations. If the V E. had not been available most of these patients would have been delivered by Cesarean section. Five of the

19 deliveries followed abandonment of forceps because of inadequate conduction anesthesia for a mid forceps manipulation. Although a second major anesthesia would have made the forceps delivery possible no additional anesthesia was needed for the vacuum extraction. Actually the V E. delivery is more effective without general or conduction anesthesia as it allows the patient to assist the expulsive force with voluntary effort.

Of the 19 cases, there were 11 positional arrests at 0 to +1 station that would have required high mid forceps for delivery a difficult and hazardous procedure at best. There were three additional cases of fetal distress after engagement and complete cervical dilatation in which the vacuum extractor was felt to be less hazardous than forceps for rapid delivery because of the relatively high station. The vacuum extractor can be successfully applied with good results in two minutes in cases of fetal distress.

The judicious use of the vacuum extractor has prevented at least 11 Cesarean sections in this series and obviated the necessity of traumatic, difficult operative vaginal deliveries in at least 19 more At the same time the maternal complications were negligible Fetal complications were acceptably low in this group There was notable reduction of the fetal complication rate in association with improvement in technique as a result of accumulation of experience with the instrument. The main example of this finding is the lessened incidence of fetal scalp abrasions. These have been one of the primary objections to the vacuum extractor *Kelly* and *Mishell* in 1962 reported a 13 per cent rate of abrasions from this institution. The present scalp abrasion rate was only 8 per cent in spite of the increased risk imposed by the more difficult situations encountered in the present series. Although 9.5 per cent of the infants were initially depressed, the incidence cannot be totally attributed to the V E. because the indication for use of the instrument in most of these cases was prolonged second stage or fetal distress.

The primary reason that the V E. has not gained acceptance in the U S may well be because of the scalp ecchymosis and artificial caput that form as part of the application However these lesions disappeared so routinely without sequelae that they are not considered a true complication. Parental acceptance of the

ecchymosis and caput was found to be no problem if the obstetrician had provided an explanation of their appearance and temporary nature before the infants were seen by the mother

As a result of this experience a concept of the role of the vacuum extractor has been evolved. Of fundamental importance is the philosophy that any indicated use of the V E. should be considered a trial of vacuum extractor Prolonged use and multiple applications of the instrument should be avoided as they may lead to unjustified risk to the fetus Intermittent traction applied for a time period greater than 30 minutes seems unwarranted. If the trial fails after a reasonable effort Cesarean section should be undertaken As a result of the high number of fetal and maternal complications in this series when trial of V E. was followed by forceps the use of forceps after the V E. is not recommended unless the V E. has advanced the vertex to a favorable position.

If forceps cannot be successfully applied, attempts to effect vaginal delivery with the V E. may then be safely undertaken. Since the V E. requires no lateral space these attempts are often successful However if the forceps have been applied successfully and traction fails it would be unwise to attempt delivery with the V E. since less traction force can be applied with this device (*Mishell* and *Kelly* 1962) Thus after failure of properly applied forceps Cesarean section should be directly performed.

The following indications are propsed for the trial of vacuum extractor

1) Positional arrest
2) Decreasing duration of labor for maternal indications (*i.e* toxemia abruptio placentae cardiac decompensation, etc.)
3) Decreasing duration of labor for fetal indications late in the first stage and in the second stage (*i e* prolapsed cord unexplained fetal bradycardia)
4) Uterine inertia unresponsive to oxytocin with nearly complete cervical dilatation in a multiparous patient
5) When general or conduction anesthesia is undesirable or unavailable and instrument delivery is indicated
6) When impossible to apply forceps correctly provided the condition of the fetus appears good.

It is hoped that the results of this study will encourage other obstetricians in this country to evaluate the place of the V E. in modern obstetrics.

SUMMARY

1) Use of the vacuum extractor over a 27 month period was critically reviewed in 168 deliveries, of which 85 per cent were indicated for obstetrical complications.
2) The vacuum extractor was used as an integral part of the clinical service and not used electively or for purposes of a clinical study
3) Significant maternal complications were absent. Fetal complications were acceptably low Corrected perinatal mortality was 1.8 per cent. Fetal scalp abrasions were only 8 per cent in this high risk group of patients
4) Cesarean section was avoided in at least 11 cases and difficult traumatic forceps deliveries avoided in at least 19 more by relatively uncomplicated vacuum extractions.
5) The concept of a trial of vacuum extractor was discussed and proposed for use in several obstetrical situations The V.E. is not advocated to replace forceps but to act as a useful companion instrument in these situations.

REFERENCES

Barr W H and *Newton M.* Am J Obst. & Gynec. *91* 403 1965
Kelly J V and *Mishell D J* Surg. Gynec. & Obst. *114* 609 1962
Malmstrom T Acta Obst et Gynec Scandinav 33 Suppl. 4 1954
Acta Obst et Gynec. Scandinav 36 Suppl. 3 1957
Acta Obst et Gynec. Scandinav 43 Suppl. 1 1964
Malmstrom T and *Jansson I* Clin. Obst & Gynec. *8* 893, 1965
McCullough C D and *Pfund B J* New York J Med. 63 2649 1963
Mishell D J and *Kelly J V* Obst. and Gynec. *19* 204 1962
Nyirjesy I Hawks, B. L Falls H C Munsat T L., and *Pierce W E.,* Am. J Obst & Gynec 85 1071 1963
Purdy J Bepko F and *Olson H* Southern Med. J. 56 1219 1963
Tricomi V Amorosi L and *Gottschalk W* Am. J. Obst. & Gynec. *81* 681 1961

Received on April 11 1967

ecchymosis and caput was found to be no problem if the obstetrician had provided an explanation of their appearance and temporary nature before the infants were seen by the mother

As a result of this experience a concept of the role of the vacuum extractor has been evolved Of fundamental importance is the philosophy that any indicated use of the V E should be considered a trial of vacuum extractor Prolonged use and multiple applications of the instrument should be avoided as they may lead to unjustified risk to the fetus Intermittent traction applied for a time period greater than 30 minutes seems unwarranted. If the trial fails after a reasonable effort Cesarean section should be undertaken As a result of the high number of fetal and maternal complications in this series when trial of V E. was followed by forceps the use of forceps after the V E. is not recommended unless the V E. has advanced the vertex to a favorable position.

If forceps cannot be successfully applied, attempts to effect vaginal delivery with the V E. may then be safely undertaken. Since the V E. requires no lateral space these attempts are often successful However,if the forceps have been applied successfully and traction fails it would be unwise to attempt delivery with the V E. since less traction force can be applied with this device (*Mishell* and *Kelly* 1962) Thus after failure of properly applied forceps Cesarean section should be directly performed.

The following indications are propsed for the trial of vacuum extractor

1) Positional arrest
2) Decreasing duration of labor for maternal indications (*i.e* toxemia abruptio placentae cardiac decompensation, etc)
3) Decreasing duration of labor for fetal indications late in the first stage and in the second stage (*i e* prolapsed cord unexplained fetal bradycardia)
4) Uterine inertia unresponsive to oxytocin with nearly complete cervical dilatation in a multiparous patient
5) When general or conduction anesthesia is undesirable or unavailable and instrument delivery is indicated
6) When impossible to apply forceps correctly provided the condition of the fetus appears good.

peutic abortion in cases where the blood pressure exceeded 160/100

Friedberg (1958) reported 13 cases of hypertensive nephropathy with 16 pregnancies which resulted in ten living infants. In the work, the object of which was to demonstrate the course of renal function during pregnancy and its restitution after deli very six deliveries were found to be complicated by toxaemia and/or uraemia. Although the author could not demonstrate any permanent deterioration in the renal disease following toxaemia of pregnancy he considered (1963) that the foetal prognosis in chronic nephritis was so poor (approximately 50 per cent foetal loss) that there were indications for therapeutic abortion in moderately severe to severe cases

In "Hypertensive disease in pregnancy *Tenney* and *Dandrow* (1961) concluded that renal disease is a most serious complica tion with poor fetal salvage This conclusion happens to be cor rect although the premises are debatable.

In another connection (*Felding*, 1964 1965) an account is given of the methods of *collecting and limiting the materials* with renal disease and pregnancy

The section of the basic material which will be analysed here consists of women under the age of 40 years with chronic renal disease the diagnosis of which had been made in hospital. Patients with diabetes were not included. Six twin deliveries and an Rh immunised patient who had two stillborn infants with erythroblastosis were also excluded from this comparison.

Nephrological data

The conditions for the diagnosis of chronic pyelonephritis were considered to be fulfilled in 68 patients. The diagnosis was established either by pathological examination of the kidney and/or according to the criteria laid down by *Bucht et al.* (1960)

Thirty-six patients were classified as having chronic glomerulonephritis without known urinary infection and without a history of pyelonephritis.

Nine patients had polycystic degeneration of the kidneys with the diagnosis confirmed by operation, radiography or autopsy

Felding, Carl, Acta obst et gynec. scandinav. 47 166 1968
From the Department of Obstetrics and Gynaecology (The late Professor Sune Genell and Professor Stig Kullander) Malmö General Hospital Sweden

THE OBSTETRIC PROGNOSIS IN CHRONIC RENAL DISEASE

BY

CARL FELDING

In previous works (*Felding*, 1964 1965 and 1967) the author demonstrated the foetal mortality prematurity and incidence of toxaemia of pregnancy in women with renal disease. The series were divided into groups urinary infections non infective renal disease post nephrectomy states congenital solitary kidney and renal disease in childhood. During the analysis it appeared that the prognosis for mother and infant was primarily dependent on the degree of renal damage and not its nature Nephropathy with hypertension appeared to be deleterious for the obstetric prognosis These problems will be investigated in the present work.

Another problem of interest in this connection is the obstetric course in subsequent pregnancies

Mackay (1963) found a relationship between increasing blood urea levels and foetal death in a series of 38 patients with various renal diseases (diabetic nephropathy polycystic degeneration, nephrocalcinosis etc.) These patients had in common blood pressures of under 175/110 Among other conclusions this author stated under "Factors affecting foetal survival *Clinical category of renal disease* It was evident that this is a significant factor The best outlook for the foetus was that in the group of patients with proteinuria as a main feature of the disease (17 per cent loss) whilst the worst was in the group with hypertensive renal disease (60 per cent)

Kaplan et al. (1962) found the maternal prognosis in hypertensive renal disease to be so poor that they proposed thera

1. the ability to concentrate was reduced to a maximum specific gravity of 1020 employing the pitressin or dehydration tests
2. there was an increase in residual nitrogen and serum creatinine and
3. there was lowered urea clearance.

The method employed varied somewhat during the years of the study and from place to place but, generally the normal upper limit of residual nitrogen was taken to be 40 mgm/100 ml, and of serum creatinine 1.2 mgm/100 ml, and the lower limit of urea clearance 80 ml/min.

The upper limit of normal blood pressure in the non-pregnant subject was established as 140/90 mm/Hg The majority of patients with hypertension had blood pressures of about 200/110 or more and values near the normal limit were only present in three patients

Hypertensive patients

Eighteen patients with 29 deliveries were recorded as being hypertensive. Of these, seven patients had chronic pyelonephritis eight chronic glomerulonephritis, two polycystic kidneys and one hydronephrosis.

Perinatal mortality 9 infants out of 29	310 ‰
Prematurity 11 infants out of 29	37 %
Severe toxaemia 12 cases out of 29	41 %

Normotensive patients

There were 22 such patients with 33 deliveries. 18 patients had chronic pyelonephritis, 2 had chronic glomerulonephritis, 1 had polycystic kidneys and 1 had hydronephrosis.

Perinatal mortality: 2 infants out of 33	60 ‰
Premature delivery 6 infants out of 33	18 %
Severe toxaemia 2 cases out of 33	6 %

Table I. *Obstetric Data*

Maternal Diagnosis	No Patients	No Deliv	Perinatal Mortality ‰		Prematurity %		Severe Toxaem
Chronic pyelonephritis	68	103	5	49 ‰	12	12 %	10
Chronic glomerulo-nephritis	36	52	8	154	7	13	9
Polycystic kidney	9	18	2	111	3	16.6	2
Renal tuberculosis	16	24	0		0		0
Hydronephrosis	4	5	1		3		1
Total	133	202	16	79	25	12	22
Malmö 1938–1960		70313	2065	29	3317	4 7	?

Sixteen patients had active or previously active renal tuberculosis (nephrectomy had been undertaken in ten)

Four patients were classified as having hydronephrosis without simultaneous evidence of pyleonephritis.

The 133 patients in the series had 202 deliveries The incidence of toxaemia of pregnancy perinatal mortality and prematurity appear in Table I The difference in the figures is not convincing and scarcely significant On the other hand the incidence of complications is greater than the overall rates during the same period of time and from the same geographical region

The significance of hypertension in relation to the obstetric prognosis

The obstetric course in the patients who showed signs of reduced renal function with or without hypertension is detailed here In most cases renal damage was demonstrated prior to pregnancy In a number of cases however reduced renal function was recorded during pregnancy or immediately after delivery indicating prepregnant chronic renal damage

Reduced renal function was considered to be present when

chemotherapy by *Kass* (1960) and *Rannevik* (1966) in the treat ment of bacteriuria are, however stimulating and will encourage increased efforts in the diagnosis of renal disease in pregnancy

An unsuccessful pregnancy in a woman with renal disease does not necessarily preclude subsequent normal deliveries although the risks of recurrence of toxaemia of pregnancy and deteriora tion in the renal disease must be regarded as considerable.

The present series is characterized by consisting entirely of women who continued their pregnancies to the stage of viability Thus, a number of women with severe renal disease for whom conception and normal pregnancy were impossible were excluded in advance.

SUMMARY

The obstetric prognosis in 202 deliveries occurring in 133 women with chronic, nondiabetic renal disease was assessed from the incidences of perinatal mortality prematurity and toxaemia of pregnancy and transient deterioration in the renal symptoms. The nephrological diagnoses were chronic pyelonephritis in 68 patients chronic glomerulonephritis in 36 polycystic kidneys in nine, renal tuberculosis in 16 and hydronephrosis in four

The total perinatal mortality was 79 pro mille, prematurity 12 per cent and toxaemia of pregnancy 10 per cent. The corre-sponding figures for 18 hypertensive patients with 29 deliveries were 310 pro mille respectively 37 and 41 per cent. The material suggests that the risk of recurrence of severe toxaemia in sub-sequent pregnancies is great but that the risk of bearing infants of less than 2500 g in subsequent pregnancies is considerably less and the incidence of repeated stillbirths is approximately the same as in a normal population.

REFERENCES

Bacht H Orrten P A and Backelin B Sv Läk. Tidn. 57 3631 1960
Dieckmann W J Toxaemias of Pregnancy Ed. 2, Mosby St Louis 1952
Felding, C Acta obst et gynec scandinav 43 141 1964
Ibidem 44 304 1935
Ibidem 46 304 1967

Although the numbers are limited the differences are striking. In agreement with the literature, this data provides evidence that the severity of the renal damage *together with hypertension* determine the obstetric prognosis

Recurrence of Obstetric Complications

Perinatal mortality Seven mothers of infants who died in the perinatal period could be followed up during at least one subsequent pregnancy Overall 11 subsequent pregnancies resulted in ten living infants

Prematurity Twenty two patients gave birth to infants weighing less than 2500 g Three of these each had one further premature infant while three others had a total of four term infants

Severe toxaemia Among the women who developed severe toxaemia of pregnancy (*Dieckmann's* classification 1952) or a corresponding deterioration in the renal disease, seven could be followed up each in one further pregnancy In each of the seven patients the symptoms recurred with transient deterioration of the condition but in all cases the symptoms regressed to the prepregnant condition during the puerperium.

This analysis suggests that a woman suffering from a renal condition who develops toxaemia of pregnancy in addition to the nephropathy runs a great risk of recurrence of the condition in subsequent pregnancies The foetal prognosis on the other hand, appears to be somewhat better

Conclusion and Discussion

This material suggests that chronic renal disease without hypertension does not exert any deleterious effect upon the obstetric prognosis The incidence of toxaemia the perinatal mortality and prematurity are more or less equal in the two largest subgroups in the series chronic pyelonephritis and chronic glomerulonephritis

The significance of bacteriuria during pregnancy cannot be assessed from this material The good results obtained with

Meyer W W Wist, A Moinian, M and Lind, J Acta obst et gynec scandinav 47 173, 1968
From the Midwifery Institute Helsinki the Wenner-Gren Research Laboratory Stockholm and Pathological Institute Mainz Western Germany

LEUCOCYTIC INFILTRATION OF UMBILICAL CORDS OBTAINED AT CAESAREAN SECTION

BY

W W MEYER, A WIST M MOINIAN AND L LIND

Leucocytic infiltration of the umbilical cord and particularly of the umbilical vein is very common in normal deliveries, but its significance and cause is still unknown. Several investigations have attributed this finding to intrauterine infection (*Bernirschke* 1962 *Pisarski et al* 1963) Other causes which have been discussed include foetal asphyxia or hypoxia (*Dominguez et al* 1960 *Widholm et al.* 1963) and venous stasis (*Beckmann* and *Zimmer* 1931) Prolonged labour (*Dominguez et al.* 1960) cord around the neck and prolapsed cord (*Bernirschke* and *Clifford* 1959) have been associated findings.

No data is available on leucocytic infiltration of umbilical cords obtained at Caesarean section, although by this means the influence of some of these factors may be excluded. This report, based on examination of a total of 164 cords, 42 obtained at Caesarean section and 122 at normal delivery therefore seems justified.

Material and Method

The cords were obtained from Caesarean sections performed during November 1966 to March 1967 in the Midwifery Institute of Helsinki (Kätilöopisto) Indications for Caesarean section

Supported in part by Expressen Prenatal Research Fund Stockholm, Semper Fund for Nutritional Research Stockholm, and by the Association for the Aid of Crippled Children, New York

Friedberg, V Zbl. Gynäk. 80 1289 1958
– Njure och graviditet Published by CIBA Stockholm 1963
Kaplan A. L. Smith J P and *Tillman A. J* Amer J Obstet. Gynec. 83 1519 1962
Kass E. H In Quinn E. L. and Kass E. H., (Eds.) Biology of Pyelonephritis J & A. Churchill LTD London 1960 p 399
Mackay E. V Aust N Z. J Obstet Gynaec. 3 21 1963
Rannevik G In Symposium on Asymptomatic Bacteriuria. Published by TIKA Ltd Umeå 1966
Tenney B and *Dandrow R. V* Amer J Obstet. Gynec. 81 8 1961

Received on May 17 1967

Author's present address
Centrallasarettet
Jönköping, Sweden

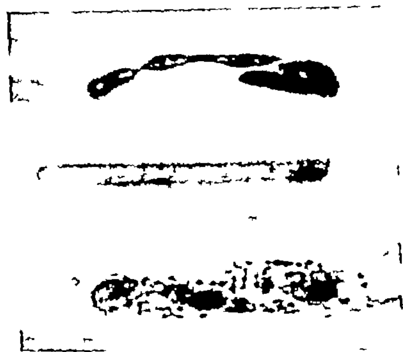


Fig 2 *Above* A migrating leucocyte in the musculature of the umbilical vein Compare its bizarre elongated nucleus with the cigarette-like nucleus of the muscle cell *Below* Goldmann staining shows dense sudanophilic granulation in the cytoplasm and permits the identification of migrating leucocytes 7933/66 Oilimmersion × 800

were made in the Kryostat. These contained all layers of the vein, as well as some Wharton's Jelly The surface area of these sections was in each case 5–8 cm^2 while the cross sectional area of the wall of the umbilical vein was only 3 mm^2 Thus, a much larger area for more thorough evaluation of the tissue was obtained. Frozen sections were also made from the cross section of the umbilical cord. Harris Hematoxylin was used for staining the sections. In several cases we used the Goldmann method (1929) with Sudan III and α-naphtol for demonstrating the presence of leucocytes in the tissue (Fig. 2)

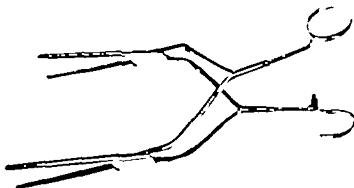


Fig 1 Double haemostat (Bunce) used in this study for clamping of umbilical cords.

included contracted pelvis placenta praevia abruptio placentae pre-eclampsia failure of cervical dilation elderly primigravida, prolonged labour prolapsed cord and foetal asphyxia. All of the mothers received 0.5 mg atropine half an hour before the operation and thiopentone 250 mg to 350 mg i.v followed by succinylcholine 75 mg before intubation. N_2O ether and O_2 inhalations were given a few minutes before delivery A low transverse incision was made in the uterus The amniotic sac was opened and then a free loop of the cord was brought out of the uterus, doubly clamped and cut. The baby was then extracted immediately The double clamp (double hemostat *Bunce* 1961) (Fig 1) with the 10 cm clamped part of the cord was put into 10 % formalin. Two tissue blocks were taken from each umbilical cord for microscopic examination 1) a cross section of the umbilical cord and 2) a piece of the outer part of the umbilical vein 6×8 to 7×10 mm in size This piece was frozen with the intima upwards and gently flattened by using a glass plate. Fifteen to twenty serial tangential sections 18–20 μ thick

Table I

	Total Number of Cases	Grade of Leucocytic Infiltration of Umbilical Cords		
		1	2	3
Caesarean section-cases	42	12	20	10
Normal deliveries	122	55	50	17

there was an infiltration of the whole wall of the vein with isolated leucocytes (*grade* 2) In the remaining cases (28 6 per cent) the changes were only classified as *grade 1* (Table I)

In the series of cords from Caesarean section babies prominent leucocytic infiltration (*grades* 2 and 3) seems to be more com mon than in those delivered vaginally but the difference is not statistically significant. In this connection it must be mentioned that Caesarean sections were performed in 24 cases before rupture of the membranes. However of 10 cases with (pronounced) infiltration (*grade* 3) Caesarean section was performed in five before rupture of the membranes in two of them before labour had not yet started. In the remaining 5 cases in which Caesarean section was done before labour started (placenta praevia (1) abruptio placentae (1) pre-eclampsia (1) contracted pelvis (2)) a leucocytic infiltration of *grade 1* or *2* was present. No signs of infection were present in either the mothers or the new born infants Even infants whose cords showed a massive leucocytic infiltration were clinically healthy

In this study Caesarean section was performed in 20 cases because of foetal asphyxia. Six of them had *grade 3* leucocytic infiltration. However of four cases with the same degree of infiltration, no asphyxia was present. Therefore asphyxia cannot be the sole cause of pronounced infiltration. No significant relationship between the grade of infiltration and other factors such as the duration of labour and the infant s weight, could be demonstrated.

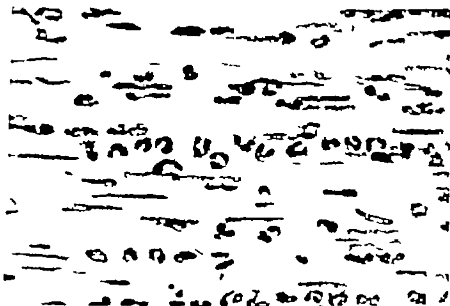


Fig 3. Pronounced phlegmon like leucocytic infiltration (grade 3) of the smooth musculature of the umbilical vein as seen in a tangential section. The nuclei of the muscle cells are cigarette-like in form Frozen section Harris-Hematoxylin. × 400

Results

A varying degree of leucocytic infiltration was found in the wall of the umbilical vein in all of the Caesarean section cords. According to the extent of this infiltration the material was divided into three grades *Grade 1* leucocytes were confined to the inner layer of the vein *Grade 2* solitary leucocytes infiltrated all layers of the umbilical vein and were occasionally present in the surrounding Wharton s jelly *Grade 3*—pronounced infiltration in both the wall of the vein and the Wharton s jelly (Fig. 3) In some of these cases the walls of the umbilical arteries were also involved.

The nuclei of the migrated leucocytes change their form markedly and unless this is taken into account the extent of leucocytic infiltration cannot be evaluated. The nature of these cells can be clearly demonstrated with appropriate staining techniques (Fig 2)

Pronounced infiltration (*grado 3*) occurred in 23 8 per cent of the Caesarean section cases In half of the cases (47 6 per cent)

moving by a small heart over a relatively long distance from the body of the foetus. Temporary functional disturbances with slowing of the blood stream may occur frequently during pregnancy causing both leucocytic infiltration of the cord and even placental lesions (fibrin deposits and infarcts) Since leucocytic infiltration has been found in all Caesarean section cases it must be considered whether this infiltration is always of pathological origin or whether it might be a physiological phenomenon of human pregnancy due to the unique organization of the foetal circulation.

SUMMARY

Microscopic examination of umbilical cords from 42 Caesarean sections, using a tangential section technique, revealed some degree of leucocytic infiltration in all cases studied. The dense, phlegmon-like infiltration was found in one-quarter of all cords examined. It was often present in the cases in which Caesarean section was performed before rupture of membranes or even before labour commenced. It is suggested that leucocytic infiltration of the cord is not caused by exogenous factors such as an ascending intrauterine infection, but is more likely to be the consequence of prenatal circulatory disturbances associated with slowing of the blood flow in the umbilical vein due to the unique organization of the extracorporeal foetal circulation

Acknowledgement

We are very grateful to Dr Arko Pathologist of Midwifery Institute of Helsinki for his generous help during this study We also wish to thank the anaesthetists and surgeons at this hospital for their active participation in this project.

REFERENCES

Beckmann S and Zimmer E Arch Gynäk 145 194 1931
Benirschke K Am J Obst & Gynec 84 1595 1962

Discussion

Pronounced leucocytic infiltration is often present in umbilical cords obtained at Caesarean section. It has been found even in cases in which Caesarean section was performed before the membranes ruptured and before the onset of labour This suggests that exogenous factors such as ascending intrauterine infection can scarcely explain this finding. On the other hand, endogenous factors may be involved and certain morphological characteristics point in this direction. Although leucocytic infiltration of the umbilical cord is often as marked as in inflammation, other signs such as oedema exudation of fibrinogen and haemorrhage are not present in the "inflamed umbilical cord. An inflammatory swelling is not seen anywhere. Even in areas with the most marked leucocytic infiltration no diffuse or localized widening of the vessel is present. Thrombosis is usually present in cases of phlebitis with a comparable degree of infiltration. But no evidence of thrombosis was found in this series. Furthermore, in spite of the marked leucocytic infiltration, the contractibility of the umbilical vessels was obviously not significantly affected because dense infiltration was seen in the walls of narrowed or closed vessels and no disintegration of muscular tissue was found.

These findings are therefore not compatible with the typical picture of inflammation caused by an infection. What is more migration of comparable degree usually takes place only in the periphery of the vascular system *i e* from the capillary This therefore appears to be a unique finding in which massive migration from a large venous vessel has taken place. Migration of leucocytes is possible particularly when the blood flow is markedly slowed. Then leucocytes accumulate in the outer layer of the blood stream and adhere to the inner surface of the vein. Thus prenatal disturbances in the foetal extracorporeal" circulation (placenta and umbilical cord) associated with temporary or repeated slowing of the blood stream could contribute to the development of leucocytic infiltration.

From a developmental point of view the extracorporeal foetal circulation is a temporary structure without nervous tissue It contains one-third of the entire blood volume of the foeto-placental system (*Yao et al* 1967) and the blood must be kept

Csapo Arpad and Sauvage, Jacques, Acta obst. et gynec. scandinav. 47 181, 1968
From the Department of Obstetrics and Gynecology (Professor Arpad I. Csapo M D) Medical School Washington University St Louis Missouri

THE EVOLUTION OF UTERINE ACTIVITY DURING HUMAN PREGNANCY[1]

BY

ARPAD CSAPO AND JACQUES SAUVAGE

Introduction

Recording intrauterine pressure in parturient patients firmly established the fact that uterine activity must reach a certain magnitude in order to initiate and promote clinical progress (*Caldeyro-Barcia* 1960 *Hendricks* 1960 *Burnhill et al.* 1962 a *Csapo et al.* 1963 a *Csapo* 1964 a) This conclusion stands irrespective of the stage of gestation, the circumstances of the initiation of labor or even the experimental conditions of the study However the methods of recording uterine activity and the analysis of the tracings being different, no general agreement has been reached as to what constitutes labor activity Yet meaningful quantitation of the intrauterine pressure and the characterization of the quality of uterine activity is mandatory if pressure tracings are to be used for diagnostic and prognostic purposes or for the assessment of the regulatory conditions of labor

The biological process through which the uterine activity of labor develops the "Evolution Process" is the subject of the present study prompted by the anticipation that the activity of labor may be better characterized by considering the nature of its evolution throughout pregnancy By this study the complex

This work has been supported by grants from the National Institutes of Health (HD01416 HD01478 and 5-K6 HD-20 169) and The Squibb Foundation

[1] Presented by Invitation of the Physiological and Obstetrical and Gynecological Societies of Lund, the Royal Caroline Institute and the Swedish Gynecological Society Stockholm Sweden May 1937

Bunce D F M Structure of the distended vascular wall Comp Ren. IVC Int. Congr Angiology Prague 708 1961
– Angiology *16* 53 1965
Dominguez R Segal A. J and *O'Sullivan J A.* J.A.M.A. *173* 346 1960
Goldmann J Zbl. Path. *46* 289 1929
Morison J E. Foetal and neonatal pathology Butterworth, London 1952
Pisarski T Breborowicz H and *Prozybora L. A.* Biol. Neonat. *5* 129 1963
Widholm O Meyer B and *v Numers C* Gynaecologia, *155* 385 1963
Yao A. Moinian M Hirvensalo M and *Lind J* In press 1967

Received on May 20 1967

Csapo, Arpad and Sauvage, Jacques, Acta obst. et gynec. scandinav. 47 181, 1968
From the Department of Obstetrics and Gynecology (Professor Arpad I. Csapo M.D.) Medical School Washington University St. Louis Missouri

THE EVOLUTION OF UTERINE ACTIVITY DURING HUMAN PREGNANCY[1]

BY

ARPAD CSAPO AND JACQUES SAUVAGE

Introduction

Recording intrauterine pressure in parturient patients firmly established the fact that uterine activity must reach a certain magnitude in order to initiate and promote clinical progress (*Caldeyro-Barcia* 1960 *Hendricks* 1960 *Burnhill et al.* 1962 a *Csapo et al* 1963 a *Csapo* 1964 a) This conclusion stands ir respecti e of the stage of gestation, the circumstances of the initiation of labor or even the experimental conditions of the study However the methods of recording uterine activity and the analysis of the tracings being different, no general agreement has been reached as to what constitutes labor activity Yet meaningful quantitation of the intrauterine pressure and the characterization of the quality of uterine activity is mandatory if pressure tracings are to be used for diagnostic and prognostic purposes or for the assessment of the regulatory conditions of labor

The biological process through which the uterine activity of labor develops, the "Evolution Process is the subject of the present study prompted by the anticipation that the activity of labor may be better characterized by considering the nature of its evolution throughout pregnancy By this study the complex

This work has been supported by grants from the National Institutes of Health (HDO1416 HDO1478 and 5-K6 HD-20 160) and The Squires Foundation

[1] Presented by invitation of the Physiological and Obstetrical and Gynecological Societies of Lund the Royal Caroline Institute and the Swedish Gynecological Society Stockholm, Sweden, May 1967

problem of the Initiation of Labor may be reduced to the relatively more simple question what factors control and by what mechanism the evolution of uterine activity with such precision, that normal labor occurs at a specific time? The present report is limited to the description in quantitative terms of the Evolution Process in normal women. Subsequent papers will describe, in similar terms the same process in rabbits and rats

The Study Patients Methods Analysis of Data and Terminology

The Patients The clinical trials to be described here were carried out in a total of 96 pregnant patients (of whom 19 were nulliparous) In this study the phase of acceleration of the Evolution Process was examined by recording uterine activity at the 36 38 40 weeks (± 1 week) of pregnancy and during spontaneous First Stage Labor A special group of patients were recorded *repeatedly* during the 36–40 weeks of gestation, to substantiate the impression that the techniques connected with recording do not affect the Evolution Process

The patients qualified for the study if they were clinically normal their prenatal case history was in agreement with their clinical status their cervices were sufficiently open for the insertion of the extraovular pressure receptor and if they volunteered for the trial. The patients were selected on the basis of these criteria from a larger group of women who desired induced labor

The stage of gestation was determined on the basis of menstrual history verified by clinical criteria and in retrospect by clinical findings at delivery The expression "Term Patient describes 40 weeks pregnant patients without distinct clinical symptoms of labor who showed no progress during a 3–4 hours observation period while the term "Early First Stage Labor describes those term patients who did have clinical symptoms of labor and did progress during the observation period but not beyond 3 cm dilatation. The expression Late First Stage Labor is reserved for term pregnant patients in spontaneous labor at 8–10 cm dilatation

Second stage labor was not studied systematically because of the high incidence of the spontaneous rupture of the fetal membranes Membrane rupture results in a leaky system and consequent errors in the accurate measurement of the intrauterine pressure. Pascal's law the physical basis of pressure studies demands *closed* rather than leaky systems for pressure measurements. The slightest leak in the recording system results in serious errors in measurements.

All study patients had intact membranes. The cervix was less than 50 per cent effaced, 1–2 cm dilated (except in those patients who were in First Stage Labor) Following the study normal pregnancy continued undisturbed, unless the patient was already in clinical labor or was induced to deliver for reasons unconnected with the study There was no complication, all patients and their newborn left the Service in good health after an average 4 days of hospitalization.

Method. A slight modification of the extraovular technique (*Csapo* 1964 a) was used for the recording of intrauterine pressure based on experiences obtained during the recording of non pregnant and pregnant women (*Csapo* and *Pinto-Dantas* 1966 a *Csapo et al* 1966 b *Csapo et al.* 1966 c *Csapo* 1964 b) During earlier trials the insertion of the recording teflon catheter and attached microballoon, above the presenting part, was somewhat hampered by the flexibility of the catheter when the cervix was less than 50 per cent effaced and 1 cm dilated. The catheter tended to bend and move sideways rather than upward during placement, as documented by X-ray evidence (*Csapo* 1965 a) The manipulations connected with correcting the poor placement of the catheter resulted in occasional rupture of the membranes. To reduce the hazard of rupturing the membranes the recording catheter was so modified (Fig 1) as to consist of a 20 cm long, rigid, (slightly bent) stainless steel tube tightly fitting the lumen of a 10 cm long (heavy walled) flexible teflon tube of 1.25 *mm* outside diameter Thus the total length of the recording catheter was 30 cm This upper flexible teflon portion of the recording catheter was protective during insertion (specifically after lubrication with antibiotic cream) while the rigid stainless steel lower portion promoted its upward movement in the extraovular space.

problem of the Initiation of Labor may be reduced to the relatively more simple question what factors control and by what mechanism the evolution of uterine activity with such precision, that normal labor occurs at a specific time? The present report is limited to the description in quantitative terms of the Evolution Process in normal women Subsequent papers will describe, in similar terms the same process in rabbits and rats.

The Study Patients Methods Analysis of Data and Terminology

The Patients The clinical trials to be described here were carried out in a total of 96 pregnant patients (of whom 19 were nulliparous) In this study the phase of "acceleration of the Evolution Process was examined by recording uterine activity at the 36 38 40 weeks (± 1 week) of pregnancy and during spontaneous First Stage Labor A special group of patients were recorded *repeatedly* during the 36–40 weeks of gestation, to substantiate the impression that the techniques connected with recording do not affect the Evolution Process

The patients qualified for the study if they were clinically normal their prenatal case history was in agreement with their clinical status their cervices were sufficiently open for the insertion of the extraovular pressure receptor and if they volunteered for the trial. The patients were selected on the basis of these criteria from a larger group of women who desired induced labor

The stage of gestation was determined on the basis of menstrual history verified by clinical criteria and in retrospect by clinical findings at delivery The expression "Term Patient describes 40 weeks pregnant patients without distinct clinical symptoms of labor who showed no progress during a 3–4 hours observation period while the term "Early First Stage Labor describes those term patients who did have clinical symptoms of labor and did progress during the observation period, but not beyond 3 cm dilatation. The expression "Late First Stage Labor is reserved for term pregnant patients in spontaneous labor at 8–10 cm dilatation.

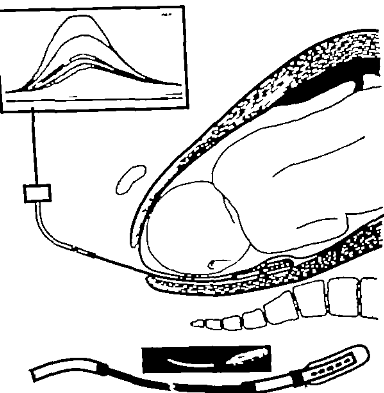


Fig 1 *Schematic illustration of the Extraovular method for the recording of intrauterine pressure* Note the position of the pressure receptor (0.8 ml microballoon, when unstretched) in the extraovular space above the presenting part The teflon and stainless steel tubes connect the pressure receptor with the pressure transducer The pressure change in the transducer is translated into an electronic signal by the transducer and appears in the recording instrument as pressure curve The set of superimposed (original) pressure cles illustrates the gradual Evolution in the shape of the pressure curves between the 36th week of gestation and 2nd stage labor

period of one hour each (1) the spontaneous activity (2) the oxytocin response at 4 at 8 and occasionally at 12 mU/min infusion rates and (3) spontaneous activity following oxytocin withdrawal The paper speed of the Sanborn (#321) was set at 5 mm min, throughout the entire study except during 10 minutes

The 4 cm long upper end of the teflon tube carried the pressure receptor a microballoon made of latex rubber This upper portion of the teflon tube, covered by the balloon was perforated at several points along its length to facilitate fluid movement between the balloon and the catheter The balloon was about 0.8 ml capacity when filled (without being stretched) with sterile water Its narrow neck was tied to the teflon tube by several loops of silk The distal end of the steel tube was connected with a Sanborn Pressure Gauge (#267BC) by a teflon catheter

The *recording catheter* with the attached microballoon was readily *inserted* in a matter of seconds into the space between the fetal membranes and the uterine wall While the patient was at bed rest in the labor room the middle finger of the examining hand entered the cervical canal and touched the lower pole of the fetal membranes The outside hand inserted the recording catheter through the vagina and the cervical canal and then into the extraovular space with the aid of the examining fingers The catheter was then gently moved upward, until the balloon passed the presenting part.

Before insertion, the recording system was made air free by overfilling it with about 3 ml sterile water and allowing the excess fluid and the air to flow out. When the balloon was in position the recording system was again overfilled with 3 ml water and the excess fluid was allowed to drip out, until the pressure became zero The balloon was the filled with up to 0 8 ml water and the "resting pressure recorded.

The patients accepted this procedure readily as a part of a *routine* vaginal examination. They also volunteered for repeated studies between the 36th and 40th weeks of gestation, when it was explained that the inducibility of labor would be examined by repeated Oxytocin Tests or when they were told that the pressure tracings would aid the attending physician in accurately determining their uterine performance The good cooperation of patients during several hours of "Monitored Labor and the ready acceptability of the method (as a routine procedure) is well reflected by the fact that over 90 per cent of the study patients were private patients of the Attending Staff

The present investigations consisted of the recording for a

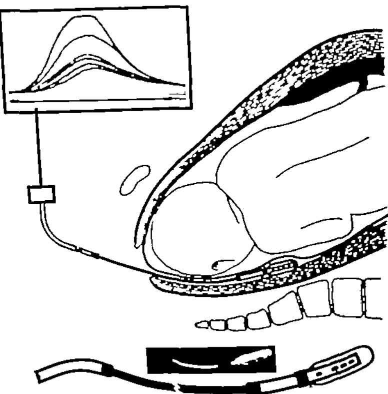


Fig 1 *Schematic illustration of the Extraovular method for the recording of intrauterine pressure* Not the position of the pressure receptor (0.8 ml nw oballoon, when unstretched) in the extraovular space above the presenting part The teflon and stainless steel tubes connect the pressure receptor with the pressure transducer The pressure change in the transducer is translated int an electronic signal by the transducer and appears in the recording instrumen as pressure curve The set of superimposed (original) pressur cycles illustrates the gradual E olution in the shape of the pressure curves between the 36th week of gestation and 2nd stage labor

period of one hour each (1) the spontaneous acti ity (2) the oxytocin response at 4 at 8 and occasionally at 12 mU/min infusion rates and (3) spontaneous activity f llowing oxytocin withdrawal The paper speed of the Sanborn (#321) was set at 5 mm min, throughout the entire study except during 10 minutes

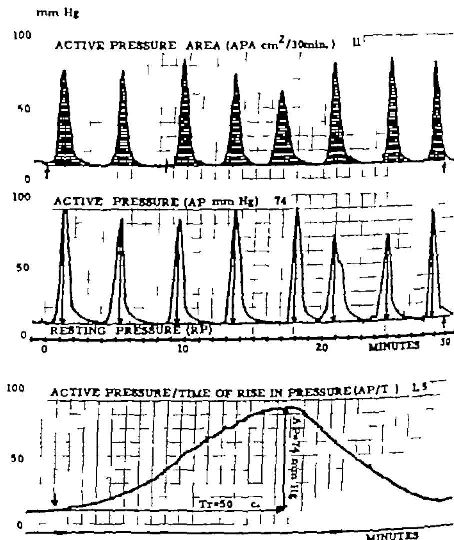


Fig. 2. *The method of analysis and terminology.* The original tracing illustrates spontaneous activity during First Stage Labor. The hatched area under the active pressure curve is the a tive pressure area, APA. It is measured by a planimeter for a period of 30 minutes (11 cm-/30 min here). The area between the baseline and the resting pressure (10 mmHg here) is not included in the measurement. The activ pressure: AP is the difference between the peak pressure and the resting pressure as indicated by the large arrow. Its average valu (at a given time) is measured by the sum of the AP values during a 30 min period, divided by the number of contractions (74 mmHg here). The average rate of rise in pressure is measured by the ratio of the AP and the time of pressure rise: AP/T (1.5 here). Extraovular technique. For further description see text.

at the end of each one hour period, when by high speed recording (20–100 mm/min) the details of the individual pressure curves were analyzed.

The analysis of the tracings (Fig. 2) included the following measurements and calculations

(1) *the average active pressure* (AP) was determined by measuring the magnitude of each single contraction cycle during a period of 30 minutes (neglecting the resting pressure which was constant at 6–12 mmHg in the present series) and dividing the sum by the number of contractions.

(2) *the active pressure area* (APAcm^2/30 min) was determin ed by measuring with a planimeter the area under the active pressure curves for a period of 30 minutes (neglecting the resting pressure)

(3) *the average rate of rise in pressure* (AP/T) was deter mined by measuring the magnitude of AP in 3 characteristic contraction cycles (in any given step of the study) dividing this value with the time needed for reaching peak pressure (T) and a eraging the 3 values

(4) the *average oxytocin response* at 8 mU/min infusion rate was determined by measuring for 30 minutes during the latter half of the 60 minutes infusion period the average AP AP/T and the APA.

The *electrophysiological terms* "local, non-propagating, asynhronic activity propagating, synchronic activity mixed local and partly propagating activity used here in describing the character of the recorded uterine activity are based on extensive earlier experiments in rabbits (*Csapo et al.* 1963 b *Csapo* and *Takeda* 1965 b) and patients (*Csapo* and *Takeda* 1963 c) In these experiments the electric activity of the uterus (at 3 distant portions) and the intrauterine pressure were simultaneously re corded *in situ* during chronic observations. As pointed out earlier (*Csapo* 1960) only the electric activity describes meaningfully nd accurately the character of uterine acti ity However once the relationship is established between the electric and mechanic

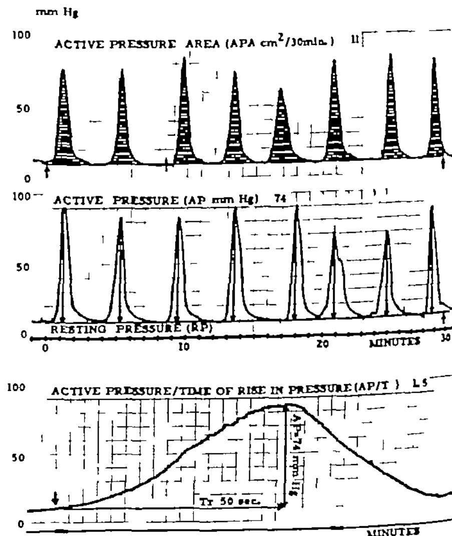


Fig 2. *The method of analysis and terminology* The original tracing illustrates spontaneous activity during First Stage Labor The hatched area under the active pressure curve is the active pressure area APA It is measured by a planimeter for a period of 30 minutes (11 cm²/30 min here) The area between the baseline and the resting pressure (10 mmHg here) is not included in the measurement. The active pressure. AP is the difference between the peak pressure and the resting pressure as indicated by the large arrow Its average value (at a given time) is measured by the sum of the AP values during a 30 min period, divided by the number of contractions (74 mmHg here) The average rate of rise in pressure is measured by the ratio of the AP and the time of pressure rise AP/T (1.5 here) Extraocular technique For further description see text.

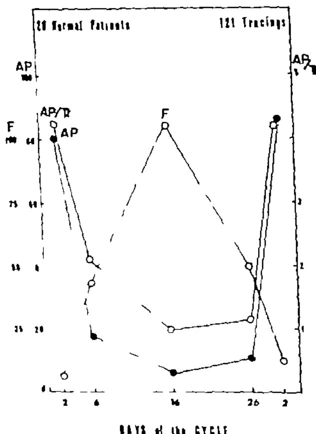


Fig 3B The graph summarizes 121 observations at cycle days 2, 6 16 26 and 2, in 20 patients Note the changes in AP AP/T and Frequency at different days of the cycle Note also that while AP and AP/T follow a similar curve Frequency has an inverse course Caspo et al

pressed, it is at day 16 of the normal cycle Furthermore the recording of uterine activity under rigorously controlled experimental conditions at days 3 6 16 1 26 and 2 in 26 normally menstruating patients yielded total of 164 tracings, confirming Figure 3B When this data was analyzed statistically by J A Hagans et al highly significant differences (P < 0.01) were found in uterine activity between day 2 and the subsequent days of the cycle but not between day 16–17 and 26 Thus while there is difference this difference is statisticall not significant

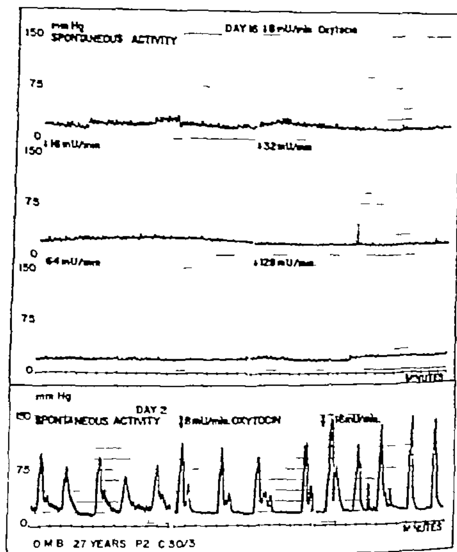


Fig. 3A. *The myometrial cycle in normal non-pregnant patients.* Original tracings illustrate the two extreme conditions of the normal myometrial cycle. Intrauterine balloon method. At day 2 (of the menstrual flow) the AP is high, the frequency is low, the oxytocin response is distinct. The shape of the pressure cycles (the high APT and the quadratic rise in pressure) indicates propagating synchronic activity. At day 16 the AP is low, the frequency is high, the oxytocin response is extinguished. The shape of the pressure cycles (the low APT and the linear or irregular rise in pressure) indicates local non-propagating, asynchronous activity.

Since the presentation of this manuscript in May 1967 Csapo and Pinto-Dantas obtained evidence that at the th week of normal pregnancy the spontaneous activity and oxytocin response of the human uterus is sup-

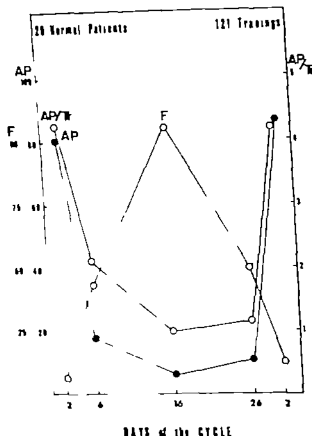


Fig 3B The graph summarizes 121 observations at cycle days 2, 6 16 26 and 2, in 20 patients Not the changes in AP AP/T and Frequency at dif ferent day of the cycle Note also that while AP and AP/T follow similar course Frequency has an inverse course Csapo et al

---

pressed, as it at day 16 of the normal cycle Furthermor the recording of uterine activity under rigorously controlled experimental conditions at days .. 3 16 17 26 and 2 in 26 normally menstruating patients yielded total of 3x4 tracings, reaffirming Figure 3B When this data was analysed statistically by I A Hagen et al highly significant differences ($P < 0.01$) were found in uterine activity between day 2 and the subsequent day of the cycle but not between days 16–17 and 26 Thus while there is difference this difference is statistically not significant

activity of the animal (*Csapo et al.* 1963 b *Csapo* and *Takeda* 1965 b) or the human (*Csapo* and *Takeda* 1963 c) uterus the mechanic activity (intrauterine pressure) becomes descriptive, at least in extreme regulatory conditions.

Results

A brief reference here to the normal *Myometrial Cycle* (*Csapo* and *Pinto-Dantas* 1966 a *Csapo et al* 1966 b) and to the uterine activity of early pregnancy (*Csapo et al* 1966 c) is necessary for the *full* description of the Evolution Process Fig 3 illustrates the 2 extreme states in uterine activity at days 2 and 17 of the normal menstrual cycle (A, original tracings) It also describes quantitatively the characteristic changes in AP AP/T and Frequency throughout the cycle in 20 patients (B) The high AP and AP/T and the oxytocin response, gradually diminish with the progressive days of the cycle reaching low values at midcycle when the oxytocin response becomes extinguished, as a rule. If pregnancy does not occur activity and oxytocin response return to the initial high values during the last day of the cycle. This rapid evolution of uterine activity is to be compared with the prolonged and gradual Evolution Process of advanced pregnancy to be described presently

Uterine activity and oxytocin response at *14 weeks* (± 1 week) of *gestation* has also been recorded (*Csapo et al.* 1966 c) as illustrated by the original tracings of Fig 4 The analysis of 5 sets of tracings (similar to that illustrated by Fig 4) revealed that spontaneous activity is drastically suppressed at the 14th week of gestation (AP = 3 mmHg, APA = 6 cm²/30 min, AP/T = 0.1) It was possible therefore, to reconstruct the uterine activity of first and second trimester pregnancy from available evidence and compare it with the data obtained in this study of late pregnancy (Figs 5 6 7)

This drastically suppressed AP of the 2nd trimester uterus 3 mmHg on the average (*Csapo et al* 1966 c *Bengtsson* and *Csapo* 1962) is of considerable theoretic interest in view of the fact that the potential activity of the uterus is significantly greater The pressure tracings revealed (*Bengtsson* and *Csapo* 1962 *Csapo et al* 1963 d *Csapo* 1966–67) that during the 14th-

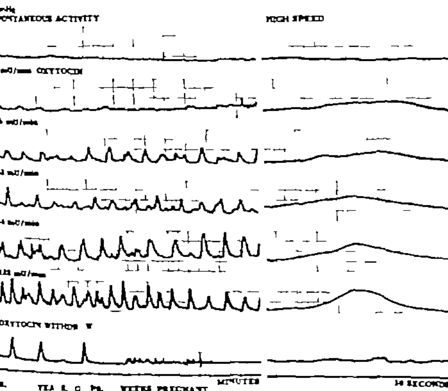


Fig. 4 The oxytocin response of the normal 14 weeks pregnant human uterus. The set of original tracings illustrates the spontaneous activity of normal 14 week pregnant human uterus before and immediately after 6 hours oxytocin infusion and the oxytocin response to 8–125 mU/min oxytocin. Transabdominal method. Cervix remained unchanged during the 6 hours period of oxytocin infusion (closed, unaffected). This study preceded the subsequent termination of pregnancy (legal abortion) with intra-amniotic hypertonic saline instillation abortion time 18.5 hours, clinical abortion 20 minutes crown foot length of the fetus 15 cm.

Note the greatly suppressed spontaneous uterine activity and the distinct but limited oxytocin response of this patient, who during the subsequent saline induced abortion displayed 60 mmHg AP. Note also the shape changes of the pressure cycles at increasing oxytocin infusion rates and the rapid decrease in activity following oxytocin withdrawal. Compare Figure 4 with Figures 3 and 5 and note the characteristic differences in oxytocin response. Consider also Footnote 1.

activity of the animal (*Csapo et al* 1963 b *Csapo* and *Takeda* 1965 b) or the human (*Csapo* and *Takeda* 1963 c) uterus the mechanic activity (intrauterine pressure) becomes descriptive at least in extreme regulatory conditions

Results

A brief reference here to the normal *Myometrial Cycle* (*Csapo* and *Pinto-Dantas* 1966 a *Csapo et al* 1966 b) and to the uterine activity of early pregnancy (*Csapo et al.* 1966 c) is necessary for the *full* description of the Evolution Process Fig 3 illustrates the 2 extreme states in uterine activity at days 2 and 17 of the normal menstrual cycle (A, original tracings) It also describes quantitatively the characteristic changes in AP AP/T and Frequency throughout the cycle in 20 patients (B) The high AP and AP/T and the oxytocin response gradually diminish with the progressive days of the cycle, reaching low values at mid-cycle when the oxytocin response becomes extinguished, as a rule If pregnancy does not occur activity and oxytocin response return to the initial high values during the last day of the cycle. This rapid evolution of uterine activity is to be compared with the prolonged and gradual Evolution Process of advanced preg nancy to be described presently

Uterine activity and oxytocin response at *14 weeks* (± 1 week) of *gestation* has also been recorded (*Csapo et al.* 1966 c) as illustrated by the original tracings of Fig 4 The analysis of 5 sets of tracings (similar to that illustrated by Fig 4) revealed that spontaneous activity is drastically suppressed at the 14th week of gestation (AP = 3 mmHg APA = 6 cm²/30 min, AP/T = 0.1) It was possible therefore, to reconstruct the uterine activity of first and second trimester pregnancy from available evidence and compare it with the data obtained in this study of late pregnancy (Figs. 5 6 7)

This drastically suppressed AP of the 2nd trimester uterus, 3 mmHg on the average (*Csapo et al* 1966 c *Bengtsson* and *Csapo* 1962) is of considerable theoretic interest in view of the fact that the potential activity of the uterus is significantly greater The pressure tracings revealed (*Bengtsson* and *Csapo* 1962 *Csapo et al* 1963 d *Csapo* 1966–67) that during the 14th-

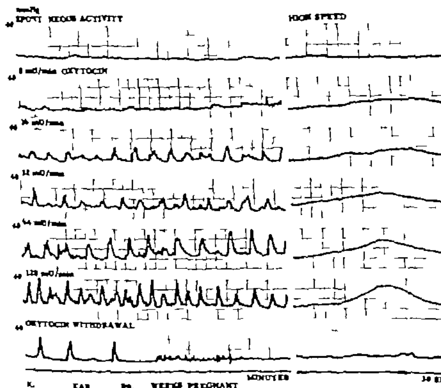


Fig. 4 *The oxytocin response of the normal 14 weeks pregnant human uterus.* The set of original tracings illustrates the spontaneous activity of normal 14 weeks pregnant human uterus before and immediately after a 6 hours oxytocin infusion and the oxytocin response to 8-128 mU/min oxytocin. Transabdominal method. Cervix remained unchanged during the 6 hours period of oxytocin infusion (closed, uneffaced). This study preceded the subsequent termination of pregnancy (legal abortion) with intra-amniotic hypertonic saline. Instillation-abortion time 18.5 hours; clinical abortion 30 minutes; crown—foot length of the fetus 15 cm.

Note the greatly suppressed spontaneous uterine activity and the distinct but limited oxytocin response of this patient, who during the subsequent saline induced abortion displayed 60 mmHg AP. Note also the shape changes of the pressure cycles at increasing oxytocin infusion rates and the rapid decrease in activity following oxytocin withdrawal. Compare Figure 4 with Figures 3 and 5 and note the characteristic differences in oxytocin response. Consider also Footnote 1.

activity of the animal (*Csapo et al* 1963 b *Csapo* and *Takeda*, 1965 b) or the human (*Csapo* and *Takeda* 1963 c) uterus, the mechanic activity (intrauterine pressure) becomes descriptive, at least in extreme regulatory conditions

Results

A brief reference here to the normal *Myometrial Cycle* (*Csapo* and *Pinto-Dantas* 1966 a *Csapo et al.* 1966 b) and to the uterine activity of early pregnancy (*Csapo et al* 1966 c) is necessary for the *full* description of the Evolution Process Fig 3 illustrates the 2 extreme states in uterine activity at days 2 and 17 of the normal menstrual cycle (A original tracings) It also describes quantitatively the characteristic changes in AP AP/T and Frequency throughout the cycle in 20 patients (B) The high AP and AP/T and the oxytocin response gradually diminish with the progressive days of the cycle, reaching low values at mid-cycle when the oxytocin response becomes extinguished, as a rule If pregnancy does not occur activity and oxytocin response return to the initial high values during the last day of the cycle. This rapid evolution of uterine activity is to be compared with the prolonged and gradual Evolution Process of advanced pregnancy to be described presently

Uterine activity and oxytocin response at *14 weeks* (±1 week) of *gestation* has also been recorded (*Csapo et al* 1966 c) as illustrated by the original tracings of Fig 4 The analysis of 5 sets of tracings (similar to that illustrated by Fig 4) revealed that spontaneous activity is drastically suppressed at the 14th week of gestation (AP = 3 mmHg, APA = 6 cm²/30 min, AP/T = 0.1) It was possible therefore to reconstruct the uterine activity of *first and second trimester pregnancy* from available evidence and compare it with the data obtained in this study of late pregnancy (Figs. 5 6 7)

This drastically suppressed AP of the 2nd trimester uterus, 3 mmHg on the average (*Csapo et al* 1966 c *Bengtsson* and *Csapo* 1962) is of considerable theoretic interest in view of the fact that the potential activity of the uterus is significantly greater The pressure tracings revealed (*Bengtsson* and *Csapo* 1962 *Csapo et al* 1963 d *Csapo* 1966–67) that during the 14th–

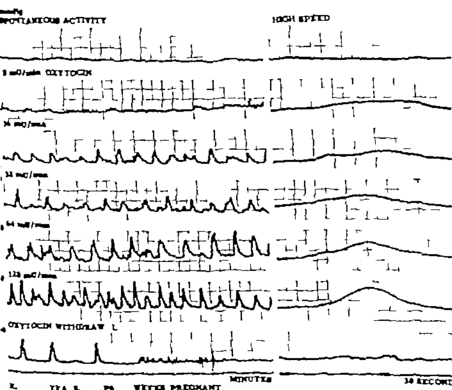


Fig 4 *The oxytocin response of the normal 14 weeks pregnant human uterus* The set of original tracings illustrates the spontaneous activity of normal 14 weeks pregnant human uterus before and immediately after a 6 hours oxytocin infusion and the oxytocin response to 8-128 mU/min oxytocin. Transabdominal method Cervix remained unchanged during the 6 hours period of oxytocin infusion (closed, uneffaced) This study preceded the subsequent termination of pregnancy (legal abortion) with intra-amniotic hypertonic saline Instillation-abortion time 18.5 hours clinical abortion 30 minutes crown—foot length of the fetus 15 cm

Note the greatly suppressed spontaneous uterine activity and the distinct but limited oxytocin response of this patient, who during the subsequent saline induced abortion displayed 60 mmHg AP Note also the shape changes of the pressure cycles at increasing oxytocin infusion rates and the rapid decrease in activity following oxytocin withdrawal Compare Figure 4 with Figures 3 and 5 and note the characteristic differences in oxytocin response. Consider also Footnote 1

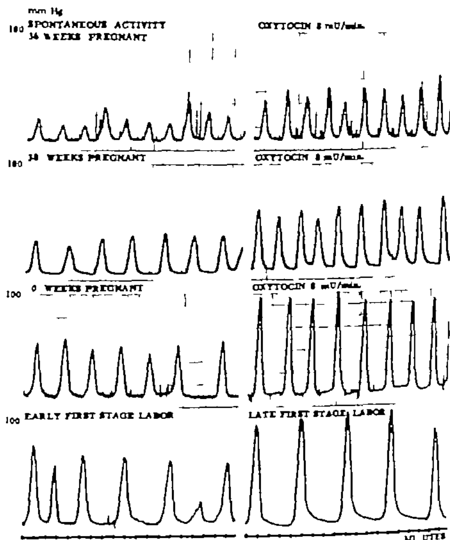


Fig 5 *The acceleration of the Evolution Process* Original tracings (Extraocular method) illustrate chara teristic changes in uterine activity and oxytocin response between the 36th week of normal pregnancy and spontaneous late First Stage Labor The tracings represent steady state conditions for the oxytocin effect rather than initi l responses. Note the gradual increase in AP and oxytocin response as gestational ge progresses

---

Fig 6 *Variations in the cha acter of activity in normal term pregnant patients* Both patients (A) and (C) are normal multiparous term pregnant patients on the verge of spontaneous labor N te the regularity in the activity of patient (A) specifically that all contraction cycles have a imilar shape and magnitude During the acceleration of activity and clinical progress (B) note









the increase in the active pressure and the rate of rise without marked changes in shape. In contrast, note the 2 types of activity at (C): the high frequency low amplitude irregular shape cycles are interrupted by low frequency high amplitude cycles showing a climbing rise in pressure (Braxton-Hicks contractions). Note also at (D) that the climbing pressure rise gradually disappears when clinical progress begins (last tracing).

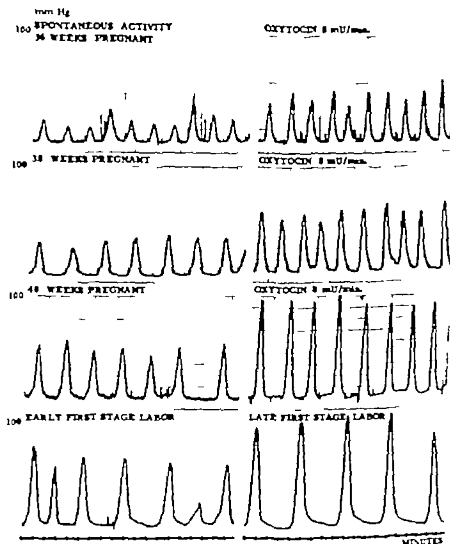


Fig 5 *The acceleration of the Evolution Process* Original tracings (Extraocular method) illustrate characteristic changes in uterine activity and oxytocin response between the 36th week of normal pregnancy and spontaneous late First Stage Labor The tracings represent steady state conditions for the oxytocin effect, rather than initial responses Note the gradual increase in AP and oxytocin response as gestational age progresses

---

Fig 6 *Variations in the character f activity in normal term pregnant patients.* Both patients (A) and (C) are normal multiparous term pregnant patients on the verge of spontaneous labor Note the regularity in the activity of patient (A) specifically that all contraction cycles have a similar shape and magnitude During the acceleration of activity and clinical progress (B) note

34th weeks of gestation, the human uterus is capable of display ing 67 mmHg average pressure (Fig. 7) when triggered to do so by hypertonic saline

The question, as to why this 67 mmHg AP is *not* normally displayed during early pregnancy was answered by studying the effect of massive oxytocin stimulation (at 64–128 mU/min in fusion rates, Fig. 4) The analysis showed (Fig. 7) that at this stage of gestation the oxytocin response is distinct, but limited to an average of 18 mmHg AP (*Csapo et al.* 1966 c *Bengtsson* and *Csapo* 1962) This is evidence that the low activity of the normal 14 weeks pregnant human uterus is not an endogenous stimulatory failure, for when massive stimulus is supplied exogenously the AP remains limited to a low value of 18 mmHg.

Fig. 5 illustrates that at the 36th *week* of gestation uterine activity is still moderate. Week by week activity increases, a fact which is readily documented by the repeated examination of the same patient, or by computing averages. The documentation is simple if the activity displayed has the type of regularity illustrated by Fig 5 or Fig. 6 A and B However it is more difficult if it has the character illustrated by Fig. 6 C and D Tracing C not only presents a problem in analysis, but also of interpretation, in that 2 types of contraction cycles are apparent, one with high frequency and low magnitude and another with low fre quency and high magnitude. These second types (the Braxton-Hicks contractions) puzzled obstetricians for some time in that clinically (by palpation) they resembled the activity of labor yet they appeared several weeks before term. A close examination of the rising phase of these pressure cycles even at low (5 mm/min) paper speed (C) but specifically at high (20 mm/min) speed (D) reveals however their climbing nature. This climbing rise in pressure distinctly different from the smooth and quadratic rise of advanced first stage labor (B or last tracing at D) is evidence that these contraction cycles result from the accidental summation of asynchronic, partly propagating, rather than synchronic propagating activity This difference in the character of the pressure rise is significant, for it reflects the lesser advanced evolutionary stage of those pressure cycles which have a climbing character Indeed while patient B was in First Stage

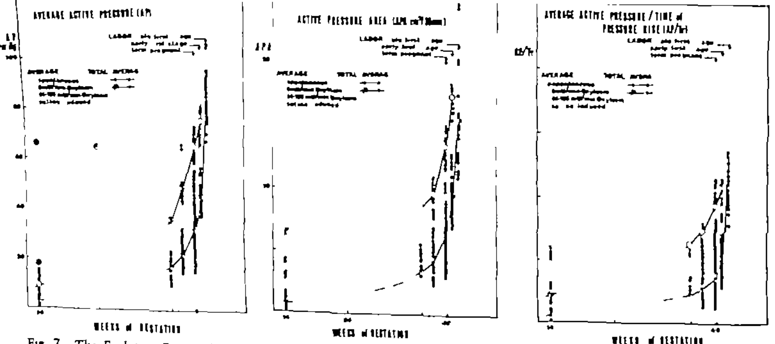


Fig 7 *The Evolution Process between the 14th week of pregnancy and Late First Stage Labor* The AP APA and AP/T are calculated from the tracings of 101 normal pregnant patients during spontaneous activity and oxytocin response (8 mU/min) At 14 weeks the response to 64–128 mU/min oxytocin is also measured (crosses) The potential activity (triangles in circles) released by the intra-amniotic injection of 20 % NaCl in 21 patients with f tal death in utero is also *illustrated* *Each circle* represents a measurement in an individual patient, pontaneous activity=filled circles oxytocin response=open circles averages=large circles with symbols inside them.

Note the gradual and limited Evolution of spontaneous activity and oxytocin esponse between th 14th and 36th week of pregnancy (measured by AP APA and AP/T) and th accelerated evolution between th 36th week of pregnancy and Early First St ge Labor Note the overall similarity in the changes in AP APA and AP/T and lso the differences N t th t the o ytocin response i not maximal at 36 weeks tha i is ery marked t 40 weeks and th t this time 8 mU/min xytocin infusion brings uterine activ ty w ll wi hin he l bor range

This distinct but limited oxytocin response of the normal pregnant human uterus between the 14th–36th weeks of gestation becomes of theoretic interest, when it is considered that the oxytocin response is extinguished during normal midcycle (*Csapo* and *Pinto-Dantas* 1966 a *Csapo et al.* 1966 b *Csapo et al.* 1966 c) and is greatly increased after fetal death in utero (*Csapo et al.* 1963 d *Csapo* 1966–67) and in those cases of normal pregnancy when the placenta turns out to be necrotic (*Bengtsson* and *Csapo* 1962) These observations indicate that in contrast to the corpus luteum the placental control of the oxytocin response of the pregnant human uterus is incomplete. The observed variations in oxytocin response during late pregnancy are additional manifestations of the incompleteness of this controlling mechanism. Further support for this interpretation is offered by the demonstration (*Burnhill et al* 1962 b) that the administration of undiluted oxytocin for several days does not induce abortion before the 18th week, while during the 2nd half of gestation massive and prolonged infusion rates at 380–710 mU/min, totalling 500–3,300 I.U (1) eventually terminated pregnancy Induction at term usually requires a total dose of 2 I.U oxytocin.

The present evidence of a limited oxytocin response at 36 weeks of pregnancy is in discord with the earlier conclusion that at the 36th week of pregnancy the myometrium has reached maximum response to oxytocin (*Caldeyro-Barcia* 1964) To what extent the method of analysis contributed to this conclusion is difficult to assess in view of the similarity in the quantitation of *spontaneous* activity of the earlier (*Caldeyro-Barcia* 1960 and 1964) and the present work

Between the *36th and 40th weeks of gestation* the Evolution Process markedly *accelerates* (Fig. 7) Normal term patients not in active clinical labor have the following average values AP = 32 mmHg, AP/T = 0.75 and APA = 6.3cm²/30 min. Apparently the Evolution in AP/T is more retarded than in AP or APA. This is an interesting finding for it suggests that propagation velocity has a delayed evolution. It is of considerable clinical importance that the oxytocin response of the 40th week pregnant uterus is dramatic, as compared to that of the normal 14–36 weeks uterus An 8 mU/min oxytocin infusion (during a one hour

Labor when the 2nd tracing (at B) was recorded, patient D only entered labor when the climbing disappeared when the pressure rise became relatively smooth and quadratic (last tracing D) A second characteristic change coincidental with the disappearance of the climbing pressure rise, is the gradual increase in the magnitude of the "local pressure cycles in between the Braxton Hicks contractions and the more frequent appearance of these contractions

Figs 5 and 6 illustrate the variations in the activity of the normal late pregnant human uterus the conceptual difficulties in interpretation and the problems of analysis But most important of all these tracings emphasize the significance of the *quality* of uterine activity reflected by the *shape* of the pressure cycles in addition to their magnitude It is the characterization of transition from local non propagating to propagating uterine activity which is considerably aided by experiences with the recording of the electric activity of the uterus (*Csapo et al* 1963 b *Csapo* and *Takeda* 1965 b *Csapo* 1960 *Csapo* and *Takeda* 1963 c) Being a *transient* phase in uterine activity the variation is so excessive between the 36th–40th weeks of gestation that descriptive terms became useless in characterizing the tracings of individual patients In contrast the term mixed local and partly propagating activity describes them all and specifically their evolutionary stage.

Of further theoretic interest is the advanced, but still *limited oxytocin response* of the uterus at 36 weeks for it emphasizes the evolutionary stage of the organ (Fig 7) By prolonged or repeated infusions at higher than 8 mU/min infusion rates uterine activity can be brought into the labor range However a one hour infusion of 8 mU/min oxytocin, which triggers 65 mmHg average AP at term (and 75 mmHg during early labor) only induces 37 mmHg average AP at the 36th week of normal gestation Thus the oxytocin responses of the 36th and the 40th week pregnant human uteri are distinctly *different* (when measured by the AP the AP/T or APA) This difference in oxytocin response is further exaggerated by giving the patient a single dose of oxytocin i.v (50 mU Oxytocin Test) rather than a prolonged infusion The results of such a study will be reported elsewhere

term pregnant women, the onset of clinical labor is not triggered by a new regulatory factor which suddenly appears at this time. The character of the curves, which describe it here, suggest that the process already begins at midtrimester

In contrast the spontaneous acceleration of activity *during* First Stage Labor is abrupt All the 3 measured parameters show this abrupt change *during* First Stage Labor in agreement with the earlier findings (*Caldeyro-Barcia* 1960 and 1964 *Hendricks* 1960) The average AP increases during First Stage Labor from 45 to 85 mmHg, the AP/T from 1 0 to 2.7 and the APA from 9.6 to 15 6cm²/30 min (Fig 7) The abruptness of these changes in uterine activity *during* active clinical labor indicates that either a new regulatory factor is now affecting uterine activity or that a factor already effective during pregnancy becomes markedly altered at this time of final acceleration, or that both of these changes occur

Discussion

This abrupt increase in uterine activity *during* labor is not without precedent. The delayed and mechanically silent but electrophysiologically distinct, Evolution of uterine activity in rabbits (Csapo and *Takeda* 1965 b) also takes an abrupt course of acceleration a few minutes before the *delivery* of the first fetus (*Fuchs* 1964 *Porter* and *Schofield* 1966) Since this abrupt *in vivo* acceleration was not seen *in vitro* and since it is similar in character to an exogenous oxytocin effect, it has been assumed (Csapo 1961 a *Fuchs*, 1964) that it is due to an endogenous oxytocin release at the time of delivery This regulatory change in rabbits is preceded by a *partial* progesterone withdrawal (*Mikhail et al* 1961) physiologically reflected by the Evolution Process which is readily and demonstrably controlled by progesterone (Csapo and *Takeda* 1965 b)

In patients the quantitative documentation of effective progesterone therapy is yet to be made but the abrupt *terminal* acceleration of the Evolution Process *during* First Stage Clinical Labor can be correlated by a partial progesterone withdrawal. Recent steroid chemical evidence, obtained by improved techniques (*Zander* 1967 *Woolever* 1965) indicates a drop in

period) triggers an AP = 65 mmHg, AP/T = 1.9 and APA = 13.5cm²/30 min. These values are quantitatively comparable to those of advanced First Stage Labor That this oxytocin induced activity at the 40th week is also qualitatively comparable to the spontaneous activity of First Stage Labor (without being identical to it) is indicated by the high success rate of "first day oxytocin inductions in normal term pregnant patients (*Page* 1943 *Theobald* 1961 *Townsend et al* 1961)

A further acceleration in Evolution occurs when the normal term patient enters clinical labor spontaneously However *in contrast* to the oxytocin stimulated term pregnant patient, the change during the spontaneous onset of labor is *not* abrupt, as a rule (*Csapo* 1964 b) Therefore the characterization of uterine activity *during* the Initiation of Labor has been unsuccessful by current analytical methods

In the present analysis of the intrauterine pressure tracings, we attempted to improve the characterization of uterine activity by computing 3 parameters rather than one and by considering the *quality* of the pressure cycles (in the AP/T) in addition to their magnitude Whether this extension of the analysis will permit us in future clinical trials to pinpoint the physiological onset of labor in serially recorded patients or further improvements in analysis will be needed for characterizing the transient state in uterine activity prior to labor remains to be determined. It is our impression that the term AP/T and the character of the rise in pressure promises considerable improvements in analysis

The present data show a considerable *overlap* in the AP AP/T and APA values of patients who are not and who are in active clinical labor The comparable average values of these 2 groups are respectively AP = 32 and 45 mmHg, AP/T = 0 75 and 1 0 APA = 6 3 and 9 6cm²/30 min While the average differences are distinct, the considerable individual variations discourage diagnostic or prognostic conclusions in case of an individual patient. An earlier study of 269 normal term pregnant women (*Csapo* 1964 b) led to a similar conclusion namely that the onset of labor in the human is physiologically undramatic, if the tracings are only analyzed in quantitative terms The gradual and continuous nature of the Evolution Process suggest that in normal

peripheral plasma progesterone *during* labor The Evolution of uterine activity is very markedly accelerated by ovariectomy placental dislocation (*Csapo* and *Takeda* 1965 b) or the intra amniotic injection of hypertonic solutions (*Csapo* 1961 b) in rabbits, or by abruptio placentae (*Csapo* 1965 a) and intra amniotic hypertonic saline treatment (*Bengtsson* and *Csapo* 1962 *Csapo et al.* 1963 d *Csapo* 1966–67) in patients The fundamental similarity as well as the superimposed modifications of the Evolution of uterine activity in rabbits, rats and patients are of sufficient theoretic interest to justify separate treatment.

The quantitative data presented here pose the questions (1) what is the regulatory background of these changes in uterine activity and (2) what is their obstetric significance?

Fig. 8 summarizes the entire course of the Evolution Process. The high uterine activity associated with the menstrual flow (showing the electrophysiological characteristics of propagating, synchronic activity) is gradually suppressed during the progressive days of the cycle until a low value is reached at the time of ovulation (showing the characteristics of local, non-propagating, asynchronic activity) If the patient becomes pregnant uterine activity does not return to its initial high value but remains suppressed, and retains the character of local, non-propagating activity

While there is some progress in Evolution during the second and early third trimester pregnancy activity remains suppressed as late in gestation as the 36th week. During all this time of suppression, the *potential* activity of the pregnant human uterus is retained at 67 mmHg (on the average) a value comparable to that of advanced First Stage Labor (Fig. 7) This potential activity is readily released in a few hours notice, at any time after the 12th week in normal pregnant patients by hypertonic saline, or in patients with fetal death in utero by a mere volume increase. In contrast, only a fraction of the potential activity is released

---

begins to increase Note around the 18th week the change in position of the curves uterine volume and placental progesterone Note specifically that the Evolution of uterine activity and oxytocin response markedly accelerate around the 36th week, the time when the increase in placental progesterone decelerates in relation to uterine volume

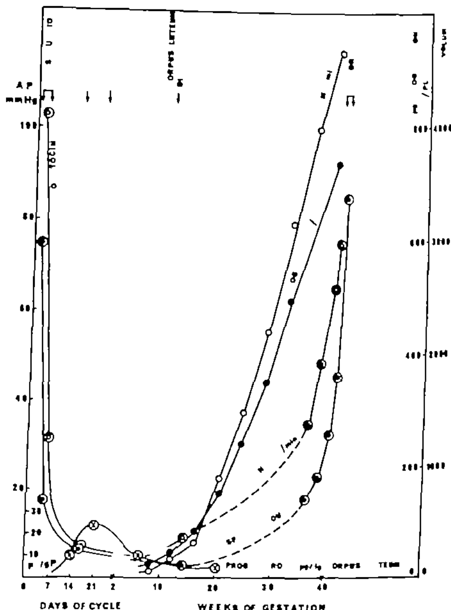


Fig 8 *A quantitative correlation in patients between uterine volume progesterone and uterine activity* The changes in the average AP during spontaneous and oxytocin induced activity are compared with the changes in corpus luteum and placental progesterone content and in uterine volume The comparison is made during the entire period between the 2nd day of the menstrual cycle and First Stage Labor Note the decrease in active pressure (AP) and oxytocin response with the advancing days of the cycle the greatly suppressed activity between midcycle and at the 14th week of pregnancy Note the reappearance of a limited oxytocin response at the 14th week. Note that when the corpus luteum progesterone declines placental progesterone

peripheral plasma progesterone *during* labor The Evolution of uterine activity is very markedly accelerated by ovariectomy placental dislocation (*Csapo* and *Takeda* 1965 b) or the intra amniotic injection of hypertonic solutions (*Csapo* 1961 b) in rabbits or by abruptio placentae (*Csapo* 1965 a) and intra amniotic hypertonic saline treatment (*Bengtsson* and *Csapo* 1962 *Csapo et al.* 1963 d *Csapo* 1966–67) in patients. The fundamental similarity as well as the superimposed modifications of the Evolution of uterine activity in rabbits, rats and patients are of sufficient theoretic interest to justify separate treatment.

The quantitative data presented here pose the questions (1) what is the regulatory background of these changes in uterine activity and (2) what is their obstetric significance?

Fig 8 summarizes the entire course of the Evolution Process. The high uterine activity associated with the menstrual flow (showing the electrophysiological characteristics of propagating, synchronic activity) is gradually suppressed during the progressive days of the cycle until a low value is reached at the time of ovulation (showing the characteristics of local, non-propagating, asynchronic activity) If the patient becomes pregnant uterine activity does not return to its initial high value but remains suppressed, and retains the character of local non-propagating activity

While there is some progress in Evolution during the second and early third trimester pregnancy activity remains suppressed as late in gestation as the 36th week. During all this time of suppression, the *potential* activity of the pregnant human uterus is retained at 67 mmHg (on the average) a value comparable to that of advanced First Stage Labor (Fig. 7) This potential activity is readily released, in a few hours notice at any time after the 12th week in normal pregnant patients by hypertonic saline or in patients with fetal death in utero by a mere volume increase. In contrast, only a fraction of the potential activity is released

---

begins to increase Note around the 18th week the change in position of the curves uterine volume and placental progesterone Note specifically that the Evolution of uterine activity and oxytocin response markedly accelerate around the 36th week, the time when the increase in placental progesterone decelerates in relation to uterine volume

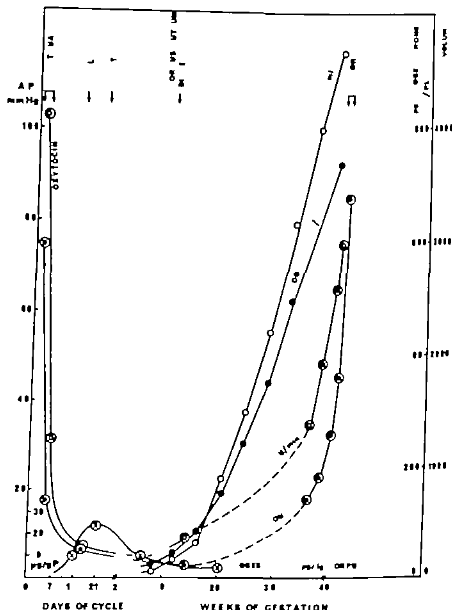


Fig. 8 *A quantitative correlation in patients between uterine volume progesterone and uterine activity* The changes in the average AP during spontaneous and oxytocin induced activity are compared with th changes in corpus luteum and placental progesterone content and in uterine volume The comparison is made during the entire period between the 2nd day of the menstrual cycle and First Stage Labor Note the decrease in active pressure (AP) and oxytocin response with the advancing days of the cycle the greatly suppressed activity between midcycl and at the 14th week of pregnancy Note the reappearance of a limited oxytocin response at the 14th week. Note that when the corpus luteum progesterone declines placental progesterone

uterus which in turn triggers the contractile response (*Csapo et al.* 1963 b *Kuriyama* and *Csapo* 1961 b)

Figure 8 illustrates that the increase in uterine volume during normal human pregnancy is very considerable specifically after the 16th week of gestation (*Rhodes* 1966) So is uterine growth (*Gillespie* 1950) but only until midterm, when the volume increase exerts an increasing stretch on the uterine wall. The extent of stretch is well reflected by the reduction in the wall thickness of the uterus from 0.9 cm (at 12 weeks) to 0.58 cm (at 40 weeks) (*Gillespie* 1950) Knowing that the pregnant human uterus at the 14th week of gestation, when its volume is only 200 ml, already has a potential of 67 mmHg AP on the average (*Csapo* 1966–67) the marked subsequent volume increase can be expected to induce a rapid Evolution in uterine activity if it is assumed that uterine volume *alone* controls the Evolution Process.

However the experimental fact is that at 4000 ml volume the normal 36 weeks pregnant uterus only displays an average 18 mmHg AP This must mean that the activity promoting effect of uterine volume (*Csapo et al.* 1963 a *Csapo et al.* 1963 b *Csapo* 1966–67) is largely (but not completely) balanced by an opposite action, retarding the Evolution Process (*Csapo* 1961 b) Progesterone being a blocking agent of the myometrium, can account for this balancing action (*Csapo* and *Takeda* 1965 b *Csapo* 1961 b) The quantitative evidence shows (Fig. 8) that around the 18th week of gestation the curves describing the increment in uterine volume (*Rhodes* 1966 *Gillespie* 1950) and placental progesterone (*Zander* 1959) change their relative positions due to a gradual *retardation* in the increase of placental progesterone This retardation becomes *marked* around the 36th week of gestation the time when the Evolution Process accelerates

Thus the quantitative evidence of various investigators (Fig. 8) is consistant with the proposal that the Evolution Process and the Initiation of Labor result from a *gradual* failure of the placenta in compensating fully through progesterone, for the activity promoting effect of uterine volume This conclusion does *not* imply that near term, or when the patient enters normal clinical

(as illustrated by Figs 7 and 8) during normal pregnancy by an 8 mU/min oxytocin infusion, which at term induces a dramatic effect. This gradually decreasing difference between the potential activity of the pregnant human uterus and its spontaneous or oxytocin induced activity is best explained by a gradually failing blocking action. The same explanation is offered here for the findings that the nearer the patient is to term the more activity is triggered by the same oxytocin infusion rate (8 mU/min) and the less likely is a first day induction failure (*Townsend et al.* 1961) Thus the quantitation of uterine activity as described here, fully confirms the conclusions based on pioneering clinical trials (*Page* 1943 *Theobald* 1961) and substantiates the clinical characterization of inducibility (*Friedman et al* 1966)

The endogenous oxytocic level of the peripheral blood of pregnant patients does not increase beyond the male value prior to advanced First Stage Labor (*Caldeyro-Barcia* 1964 *Couthino* 1964) Following fetal death in utero when the estrogens drop markedly (*Cassmer* 1948) labor may begin within a few hours or only after several months Thus the correlation between the Initiation of Labor and oxytocics or estrogens is poor Therefore among the regulatory agents whose *direct* effect on the uterus is thoroughly documented two factors uterine volume and progesterone remain as likely dominant, controlling agents of the Evolution Process and the Initiation of Labor The phrase dominant stands here to indicate that various other fetal and maternal regulatory factors are implicated in the control of the endogenous time clock of the Initiation of Labor whose actions however are either indirect or moderate.

The increase in *uterine volume* (V) during pregnancy must be considered as a powerful physical parameter of uterine activity on 3 accounts

(1) through chronic stretch it induces myometrial hypertrophy (*Reynolds* 1949 *Csapo et al* 1965 c)

(2) through the "length tension relationship it controls the *contractile* response of the uterus (*Csapo* 1960)

(3) through the effect of stretch on *threshold* (*Kuriyama* 1961 a) it controls the threshold relation (*Csapo* 1960) and thus the generation and propagation of the electric activity of the

same degree as that observed after hypertonic saline treatment in midtrimester patients.

It would appear therefore, that the potential activity of the pregnant human uterus is primarily sustained, in increasing readiness, by the gradually increasing uterine volume, an effect to which the ovarian and placental steroids contribute. Ovarian and later placental progesterone balance this activity promoting effect of uterine volume, through a blocking action. The degree of the block seems to be controlled by a number of factors such as the ratios ovarian/placental progesterone, non-placental/placental uterine regions, rate of progesterone synthesis progesterone transport, metabolism, etc. The difference between the potential and the *de facto* activity of the pregnant uterus, the rate of the Evolution of uterine activity seem to reflect the compromise between the increasing stimulus of uterine volume and the gradually falling blocking action. Thus the theory V/P does not suggest that the Initiation of Labor results from a marked progesterone withdrawal, but rather from the failure of the placenta in compensating *fully* for the activity promoting effect of uterine volume. This proposed compensatory potential of the placenta is presently investigated in early pregnant patients whose uterine volume is increased experimentally

The activity required for the Initiation of Labor is quantitatively defined here by the average values 45 mmHg AP 1 0 AP/T 9 6cm²/30 min APA and 9 6 contractions/30 min frequency While these average values are meaningful in that they describe the majority of patients they have the shortcoming of offering no explanation for a considerable minority who fail to enter labor at higher than average values. The possibility that various combinations of the terms AP AP/T APA and fre-

Since the presentation of this manuscript, M. Pulkkinen and A. Kärkkäinen of Turku Finland studied the compensatory reserve or potential of the placenta during volume induction (with isotonic saline) in 4 normal midtrimester patients As predicted (by A.C.) only 1 out 24 patients aborted after an average volume increase of 431 ± 17 ml The remaining 23 patients only showed transient increase in activity finding consistent with the concept that the volume increase may be compensated for by placental progesterone reserve or transiently increased synthesis.

labor a "progesterone withdrawal does occur Only a *relative* progesterone *deficiency* is proposed on the basis of experimental evidence Whether a partial progesterone withdrawal occurs before during, or only shortly after the Initiation of Labor would seem difficult to determine, in view of the magnitude of the ex pected change and the variations in the plasma progesterone concentrations published by different investigators (*Zander* 1959 1967 *Short* and *Eton* 1959 *Wiest* 1967) However the degree of a *partial* progesterone withdrawal is not decisive in theoretic considerations for the apparent *relative* progesterone deficiency (Fig 8) and the fact that among all the known regulatory com pounds progesterone is the only one which has been isolated from the myometrium (*Wiest* 1967 *Kumar* and *Barnes* 1965) underline the regulatory importance of this hormone in the Initiation of Labor

The concept of a *relative progesterone deficiency* (*Csapo* 1961 b) only incompletely balancing the activity promoting effect of uterine volume was derived from model experiments in rabbits (*Csapo et al* 1963 *Csapo* and *Takeda* 1965 b) and from clinical trials on hypertonic saline induction (*Csapo* 1966–67) The relationship of these two regulatory factors volume (V) and progesterone (P) was expressed by the ratio V/P The theory V/P was substantiated by the recent demonstrations (see for references (*Csapo* 1966–67)) that hypertonic saline treatment not only increases V but also decreases P (by 33 per cent prior to the onset of clinical abortion) that when after fetal death in utero P gradually decreases a mere increase in V triggers uterine activity and abortion and that in normal term patients (relative progesterone deficiency) an increase in V also precipitates labor as a rule while during early pregnancy it does not. It is the exogenous effect of progesterone in the pregnant human uterus where the documentation is as yet unsatisfactory (*Csapo et al.* 1966 d) but it is doubtful whether the myometrial progesterone concentration can be *increased significantly* by the therapeutic doses of systematic progesterone However the theory is supported by the recent finding (*Zander* 1967 *Woolever* 1965) that during First Stage Labor when uterine activity markedly accelerates there is a partial progesterone withdrawal of the

same degree as that observed after hypertonic saline treatment in midtrimester patients.

It would appear therefore, that the potential activity of the pregnant human uterus is primarily sustained, in increasing readiness by the gradually increasing uterine volume an effect to which the ovarian and placental steroids contribute. Ovarian and later placental progesterone balance this activity promoting effect of uterine volume through a blocking action. The degree of the block seems to be controlled by a number of factors such as the ratios ovarian/placental progesterone non-placental/placental uterine regions, rate of progesterone synthesis, progesterone transport, metabolism, etc. The difference between the potential and the *de facto* activity of the pregnant uterus the rate of the Evolution of uterine activity seem to reflect the compromise between the increasing stimulus of uterine volume and the gradually failing blocking action. Thus the theory V/P does not suggest that the Initiation of Labor results from a marked progesterone withdrawal, but rather from the failure of the placenta in compensating *fully* for the activity promoting effect of uterine volume This proposed compensatory potential of the placenta is presently investigated in early pregnant patients whose uterine volume is increased experimentally

The activity required for the Initiation of Labor is quantitatively defined here by the average values 45 mmHg AP 1 0 AP T 9 6cm²/30 min APA and 9 6 contractions/30 min frequency While these average values are meaningful in that they describe the majority of patients they have the shortcoming of offering no explanation for a considerable minority who fail to enter labor at higher than average values The possibility that various combinations of the terms AP AP/T APA and fre-

Since the presentation of this manuscript, M Pulkkinen and A. Kivikoski of Turku Finland studied the compensatory reserve or potential of the placenta during volume induction (with isotonic saline) in 24 normal midtrimester patients As predicted (by A.C) only 1 out 24 patients aborted after an average volume increase of 431 ± 17 ml The remaining 23 patients only showed transient increase in activity a finding consistent with the concept that the volume increase may be compensated for by placental progesterone reserve or transiently increased synthesis.

quency improve their diagnostic and prognostic meaning has not yet been studied systematically The group of patients presenting the most serious difficulties in the interpretation of the uterine pressure tracings is the one which is induced by oxytocin before or at term, and fail in delivery during first day induction in spite of their greater than average total induced activity This failure is evidence that the quantity of activity alone is an inadequate measure of effective uterine effort.

Thus the *quality* of activity emerges as a crucial obstetric parameter It has been proposed (*Caldeyro-Barcia* 1964) that the discrepancy between induced activity and clinical progress is due to differences in cervical resistance. However it is doubtful whether cervical resistance can account for first day induction failures since the most unfavorable (closed and uneffaced) cervices of midtrimester patients were shown to yield readily and predictably to *that type* of activity which is induced by hypertonic saline (*Bengtsson* and *Csapo* 1962 *Csapo et al* 1963 d *Csapo* 1966–67) Thus the clinical finding, that patients induced (Rh incompatibility) by the combined treatment of rupturing the membranes and oxytocin infusion during the 38–40 weeks are successfully delivered in 70 per cent of the cases during the first oxytocin treatment whereas those 32–35 weeks pregnant are only delivered in 30 per cent of the cases must be explained by other variables than cervical resistance.

The meaning of the term *quality* may be clarified by the following example Let us assume that uterine activity effectively dilates the cervix if it has a definite direction, *i.e* if it originates in the fundal region (*Reynolds* 1949) and from there it propagates *downward*. If oxytocin treatment *simultaneously* depolarizes different uterine regions the coincidental decrease in the threshold most probably generates activity at *multiple* points simultaneously Such an action could induce high activity without the quality of gradual propagation in a definite direction and therefore may be clinically ineffective. This illustration of a point is of course speculative. However it is not entirely without experimental ground. The marked increase around the 36th week of pregnancy induced by oxytocin infusion, in the AP/T without a comparable increase in AP can be interpreted as an expression

of multiple, simultaneous, partial and directionless activation.

The quality of uterine activity the extent, velocity and direction of propagation, can be characterized by recording electric activity at multiple points directly from the myometrium. This is possible and has been done with success (*Csapo* and *Takeda* 1965 b *Csapo* 1960 *Csapo* and *Takeda* 1963 c) but the procedure is not suitable for routine, clinical use. Electrohysterography using external electrodes, resolves the problem of routine application but yields complex tracings whose interpretation is difficult (*Sureau* 1955) Therefore, the techniques must be further improved before uterine activity in patients can be meaningfully characterized in electrophysiological terms and before a correlation can be documented between the location of the placenta, the character of uterine activity and clinical progress Until these technical advances are realized it would seem advisable to withhold judgment about the regulatory and clinical significance of placental implantation site.

SUMMARY

The quantitative experimental evidence obtained by sensitive and accurate physiological methods in normal patients, demonstrated that

(1) Uterine activity changes characteristically between the 2nd day of the menstrual cycle and 2nd stage labor in the form of a U shaped curve When these changes are analyzed, the

Since the presentation of this manuscript Csapo, *Sauvage* *Pulkkinen* and Wood examined the relationship between placental location and the duration of oxytocin induced monitored labor in 143 normal term patients, under rigorously controlled experimental conditions In this study (in contrast to earlier ones) placental location has been determined by 3 (rather than 1) independent techniques and the onset of labor was sharply defined by the moment of oxytocin infusion Patients with "high-fundal" implantation delivered in 345 ± 21 min while those with middle-low implantation in 242 ± 11 min This difference is very highly significant statistically (P < 0 001) This study also provided an explanation for the failure of others in demonstrating this relationship Our detailed analysis showed that it is the duration of the early phase of labor (up to 3 cm dilatation) which is most markedly affected by placental location, the phase which has not been considered by others, due to their definition of clinical onset

quency improve their diagnostic and prognostic meaning has not yet been studied systematically The group of patients presenting the most serious difficulties in the interpretation of the uterine *pressure tracings* is the one which is induced by oxytocin before or at term and fail in delivery during first day induction, in spite of their greater than average total induced activity This failure is evidence that the quantity of activity alone is an inadequate measure of effective uterine effort.

Thus the *quality* of activity emerges as a crucial obstetric para meter It has been proposed (*Caldeyro-Barcia* 1964) that the discrepancy between induced activity and clinical progress is due to differences in cervical resistance However it is doubtful whether cervical resistance can account for first day induction failures since the most unfavorable (closed and uneffaced) cer vices of midtrimester patients were shown to yield readily and predictably to *that type* of activity which is induced by hyper tonic saline (*Bengtsson* and *Csapo* 1962 *Csapo et al.* 1963 d *Csapo* 1966–67) Thus the clinical finding, that patients induced (Rh incompatibility) by the combined treatment of rupturing the membranes and oxytocin infusion during the 38–40 weeks are successfully delivered in 70 per cent of the cases during the first oxytocin treatment, whereas those 32–35 weeks pregnant are only delivered in 30 per cent of the cases must be explained by other variables than cervical resistance.

The meaning of the term *quality* may be clarified by the following example. Let us assume that uterine activity effectively dilates the cervix if it has a definite direction i.e if it originates in the fundal region (*Reynolds* 1949) and from there it propagates *downward*. If oxytocin treatment simultaneously depola rizes different uterine regions the coincidental decrease in the threshold most probably generates activity at *multiple* points simultaneously Such an action could induce high activity without the quality of gradual propagation in a definite direction and therefore may be clinically ineffective. This illustration of a point is of course speculative. However it is not entirely without ex perimental ground The marked increase around the 36th week of pregnancy induced by oxytocin infusion, in the AP/T without a comparable increase in AP can be interpreted as an expression

of multiple, simultaneous partial and directionless activation.

The quality of uterine activity the extent, velocity and direction of propagation, can be characterized by recording electric activity at multiple points directly from the myometrium. This is possible and has been done with success (*Csapo* and *Takeda* 1965 b *Csapo* 1960 *Csapo* and *Takeda* 1963 c) but the procedure is not suitable for routine, clinical use. Electrohysterography using external electrodes resolves the problem of routine application but yields complex tracings whose interpretation is difficult (*Sureau* 1955) Therefore, the techniques must be further improved before uterine activity in patients can be meaningfully characterized in electrophysiological terms and before a correlation can be documented between the location of the placenta, the character of uterine activity and clinical progress Until these technical advances are realized it would seem advisable to withhold judgment about the regulatory and clinical significance of placental implantation site.

SUMMARY

The quantitative experimental evidence, obtained by sensitive and accurate physiological methods in normal patients demonstrated that

(1) Uterine activity changes characteristically between the 2nd day of the menstrual cycle and 2nd stage labor in the form of a U shaped curve. When these changes are analyzed, the

Since the presentation of this manuscript Csapo *Sauvage* *Pulkkinen* and Wood examined the relationship between placental location and the duration of oxytocin induced monitored labor in 143 normal term patients, under rigorously controlled experimental conditions. In this study (in contrast to earlier ones) placental location has been determined by 3 (rather than 1) independent techniques and the onset of labor was sharply defined by the moment of oxytocin infusion. Patients with "high-fundal" implantation delivered in 345 ± 21 min while those with middle-low implantation in 242 ± 11 min This difference is very highly significant statistically ($P < 0.001$) This study also provided an explanation for the *failure* of others in demonstrating this relationship. Our detailed analysis showed that it is the duration of the early phase of labor (up to 3 cm dilatation) which is most markedly affected by placental location, the phase which has not been considered by others, due to their definition of clinical onset

curves retain the U shape on a 40 weeks time scale irrespective of whether AP APA or AP/T are being computed, indicating similar underlying functional and regulatory backgrounds for these expressions of uterine activity However a closer comparison of the 3 U shaped curves show modifications in shape indicating that while these 3 terms, AP APA and AP/T have a common background, they are different expressions of myometrial function. The corresponding changes in the frequency of cyclic activity are more complex. The shape of the curves describing them depends on whether the analysis includes pressure cycles of 10 5 3 mmHg or less.

(2) The left leg of the U shape curve describes the deceleration of uterine activity the right leg its acceleration (the Evolution Process) while the connecting bottom portion corresponds with the block The high activity at the extreme left and right of the U has the character of propagating synchronic activity the suppressed bottom portion, local non propagating, asynchronic activity while the intermediates between these extreme states are mixtures of the two This terminology is not arbitrary but is based on electrophysiological evidence

(3) Both legs of the U shaped curve can be shifted, on the time scale by physiological (or near physiological) oxytocin infusions. The slight shift of the left leg is evidence that deceleration can only be delayed to a limited extent while the marked shift of the right leg to the left illustrates that acceleration can be hastened considerably by exogenous control. Under similar experimental conditions the bottom of the U curve can not be lifted significantly This is further evidence for the 3 different regulatory conditions of the human uterus already described at (2)

(4) The regulatory background of this U shaped curve is explained to a large extent by known and quantitatively described variations in uterine volume and progesterone during pregnancy The deceleration in activity and the extinction of the oxytocin response is best accounted for by a gradually developing systemic progesterone block If this block is withdrawn due to the short life span of the corpus luteum of non pregnancy a rapid Evolution of uterine activity follows culminating in the onset of the

next menstrual cycle. If on the other hand a corpus luteum of pregnancy develops and is maintained, the block is sustained and when corpus luteum function shifts to the placenta, the character of the block is modified. The gradual and continuous nature of the Evolution in spontaneous activity and oxytocin response during pregnancy reflect the incompleteness and gradually increasing failure of the placental block.

(5) The blocked uterus of pregnancy has a working potential comparable to the parturient uterus. This potential originates to a large extent, from the gradually increasing uterine volume affecting the pregnant myometrium in concert with the ovarian steroids (and those of the placenta) The profound increase in uterine volume during normal pregnancy and the well documented coincidental hypertrophy fully account for the measured working potential as well as for the initiation of activity of the pregnant human uterus. There is no need for assuming an oxy tocic agent in the promotion of the Evolution Process and the experimental evidence shows that such an agent does not appear in the bloodstream, when as a result of advanced Evolution, labor is initiated.

(6) The powerful activity promoting effects of increasing uterine volume are only incompletely balanced by the compen satory efforts of the placenta in accelerating progesterone synthesis The quantitative evidence shows a gradual failure in pla cental compensation, which becomes marked at around the 36th week of normal gestation. The initiation of labor is explained here by the acceleration of the Evolution Process due to a relative progesterone deficiency resulting from the failure of the placenta in compensating fully for the the activity promoting effect of increasing uterine volume

(7) The clinical significance of the presented quantitative physiological data is discussed.

Acknowledgment

The authors are grateful to Professor Willard M. Allen, the Chairman of the Dept., for his full support during these clinical trials and to the Attendings of our Service specifically Drs.

curves retain the U shape on a 40 weeks time scale irrespective of whether AP APA or AP/T are being computed, indicating similar underlying functional and regulatory backgrounds for these expressions of uterine activity However a closer comparison of the 3 U shaped curves show modifications in shape, indicating that while these 3 terms AP APA and AP/T have a common background, they are different expressions of myometrial function. The corresponding changes in the frequency of cyclic activity are more complex. The shape of the curves describing them depends on whether the analysis includes pressure cycles of 10 5 3 *mmHg*, or less

(2) The left leg of the U shape curve describes the deceleration of uterine activity the right leg its acceleration (the Evolution Process) while the connecting bottom portion corresponds with the block. The high activity at the extreme left and right of the U has the character of propagating synchronic activity the suppressed bottom portion local non-propagating, asynchronic activity while the intermediates between these extreme states are mixtures of the two This terminology is not arbitrary but is based on electrophysiological evidence

(3) Both legs of the U shaped curve can be shifted on the time scale by physiological (or near physiological) oxytocin infusions The slight shift of the left leg is evidence that deceleration can only be delayed to a limited extent, while the marked shift of the right leg to the left illustrates that acceleration can be hastened considerably by exogenous control. Under similar experimental conditions the bottom of the U curve can not be lifted significantly This is further evidence for the 3 different regulatory conditions of the human uterus already described at (2)

(4) The regulatory background of this U shaped curve is explained to a large extent by known and quantitatively described variations in uterine volume and progesterone during pregnancy The deceleration in activity and the extinction of the oxytocin response is best accounted for by a gradually developing systemic progesterone block If this block is withdrawn due to the short life span of the corpus luteum of non pregnancy a rapid Evolution of uterine activity follows culminating in the onset of the

Csapo A. I and *Takeda H* Electrical Activity of the Parturient Human Uterus, Nature 200 680 1963

Csapo A. I *Jaffin H* *Kerenyi T* *de Mattos C. E. R.* and Souza-Filho M B Fetal Death in Utero Am. J Obst. & Gyn. 87 892, 1953 d

Csapo A I Extraovular Pressure—Its Diagnostic Value Am. J Obst. & Gyn. 90 493 1964

Csapo A The Intra-Uterine Control of Parturition. Second International Congress of Endocrinology International Congress Series No. 83 London 1964 b

Csapo A. The Placenta and the Initiation of Labor Nederlandsch Tijdschrift voor Verloskunde en Gynaecologie 65 229 1965 a

Csapo A. I and *Takeda H* Effect of Progesterone on the Electric Activity and Intrauterine Pressure of Pregnant and Parturient Rabbits Am. J Obst. & Gyn 91 221 1965 b

Csapo A Erdos T De Mattos C R Gramss E and Moscowitz C Stretch-Induced Uterine Growth Protein Synthesis and Function, Nature 207 1378 1965

Csapo A I and Pinto-Dantas C A The Cyclic Activity of the Non-Pregnant Human Uterus Fert & Ster 17 34 1966

Csapo A I Pinto-Dantas C A and Kerenyi T Progesterone and Myometrial Activity in press from Fifth World Congress on Fertility and Sterility Stockholm, 1966 b

Csapo A I *Kerenyi T* *De Souza-Filho M B*, *Pinto-Dantas C A*, *De Souza O* *Darne E* and Ogata S Control of the Lengths of the Menstrual Cycle and Gestation. Presented at Symposium of the Society for the Study of Fertility Cambridge, 1966

Csapo A *De Souza-Filho M B* *De Souza J C* and *De Souza O* Effect of Massive Progestational Hormone Treatment on the Parturient Human Uterus Fert & Ster 17 621 1966 d

Csapo A The Termination of Pregnancy by the Intra-Amniotic Injection of Hypertonic Saline (1966–1967 Year Book of Obstetrics & Gynecology) Ed J P Greenhill Year Book Medical Publishers

Friedman E Niswander K R Bayonet-Rivera N P and Sachtleben M R Relation of Prelabor Evaluation to Inducibility and the Course of Labor Obst & Gynec 28 495 1966

Fuchs A R Oxytocin and the Onset of Labour in Rabbits *J* Endocrin 30 217 1964

Gillespie E C Principles of Uterine Growth in Pregnancy Am. J Obst. & Gy 59 949 1950

Hendricks C H Comments on the Prevention of Prematurity PHYSIOLOGY OF PREMATURITY Trans. Fifth Conf ed. M. Kowlessar Spons. by Josiah Macy Jr Foundation 1960

Kumar D and Barnes A C Studies in Human Myometrium During Pregnancy Article 6 Tissue Progesterone Profile of the Various Compartments in the Same Individual Am J Obst & Gyn 92 717 1965

C R Gulick F B Long, N K. Muschany and J B Martin for stimulating discussions and their cooperation in providing us with the study patients. Our special thanks are due to research nurse Judy Gamblin and Carole English for their dedicated attention of the patients and Elise Gramss and Vivian Hertelendy for the analysis of the tracings

REFERENCES

Bengtsson L. Ph and *Csapo A. I* Oxytocin Response Withdrawal and Reinforcement of Defense Mechanism of the Human Uterus at Mid-pregnancy Am. J Obst. & Gyn. *83* 1083 1962

Burnhill M S Daniels J and *Cohen J* Uterine Contractility During Labor Studied by Ultra-Amniotic Fluid Pressure Recordings Am. J Obst. & Gyn. *83* 561 1952 a

Burnhill M Gaines J and *Guttmacher A.* Concentrated Oxytocin Solution for Therapeutic Interruption of Midtrimester Pregnancy Obst. & Gynec. *20* 94 1962 b

Caldeyro-Barcia R. Factors Controlling the Action of the Pregnant Human Uterus PHYSIOLOGY OF PREMATURITY Trans Fifth Conf ed. M. Kowlessar Spons by Josiah Macy Jr Foundation 1960

Caldeyro-Barcia R. Regulation of Myometrial Activity in Pregnancy MUSCLE, Proc Symposium at the Faculty of Medicine, Univ of Alberta 1964 Eds W M. Paul E. E Daniel C M. Kay & G Monckton, Pergamon Press

Cassmer O Hormone Production of the Isolated Human Placenta. Studies on the Role of the Foetus in the Endocrine Function of the Placenta, Acta Endocrin. Suppl *45* 1948

Couthino E M Hormone Induced Ionic Regulation of Labor Second International Congress f Endocrinology International Congress Series No 83 London 1964

Csapo A The Asymmetrical Uterus and the Mechanism of Parturition PHYSIOLOGY OF PREMATURITY Trans Fifth Conf ed. M Kowlessar Spons by Josiah Macy J Foundation 1960

Csapo A The Effects of Oxytocic Substances on the Excitability of the Uterus OXYTOCIN—AN INTERNATIONAL SYMPOSIUM Pergamon Press 1961 a

Csapo A. On Progesterone and the Defence Mechanism of Pregnancy Ciba Foundation Study Group No 9 1961 b

Csapo A. I Jaffin H Kerenyi T Lipman J and *Wood C* Volume and Activity of the Pregnant Human Uterus Am. J Obst. & Gyn. *85* 819 1963 a

Csapo A. I Takeda H and *Wood C* Volume and Activity of the Parturient Rabbit Uterus Am. J Obst & Gyn *85* 813 1963 b

Csapo A. I and Takeda H Electrical Activity of the Parturient Human Uterus Nature 200 680 1963 c

Csapo A. I Jaffin H Kerenyi T de Mattos C. E. R. and Souza-Filho M B Fetal Death in Utero, Am. J Obst. & Gyn. 87 892, 1963 d

Csapo A. I. Extraovular Pressure—Its Diagnostic Value Am. J Obst. & Gyn. 90 493 1964 a

Csapo A. The Intra-Uterine Control of Parturition. Second International Congress of Endocrinology International Congress Series No 83 London 1964 b

Csapo A The Placenta and the Initiation of Labor Nederlandsch Tijdschrift voor Verloskunde en Gynaecologie 65 229 1965 a

Csapo A. I. and Takeda H Effect of Progesterone on the Electric Activity and Intrauterine Pressure of Pregnant and Parturient Rabbits Am. J Obst. & Gy 91 221 1965 b

Csapo A Erdos T De Mattos C. R Gramss E and Moscowitz C Stretch-Induced Uterine Growth Protein Synthesis and Function Nature 207 1378 1965

Csapo A I and Pinto-Dantas C A. The Cyclic Activity of the Non-Pregnant Human Uterus Fert. & Ster 17 34 1966

Csapo A I Pinto-Dantas C A. and Kerenyi T Progesterone and Myometrial Activity in press from Fifth World Congress on Fertility and Sterility Stockholm, 1966 b

Csapo A I Kerenyi T De Souza-Filho M. B Pinto-Dantas C A De Souza O Derse E and Ogata S Control of the Lengths of the Menstrual Cycle and Gestation, Presented at Symposium of the Society for the Study of Fertility Cambridge 1966

Csapo A De Souza-Filho M. B De Souza J C. and De Souza O Effect of Massive Progestational Hormone Treatment on the Parturient Human Uterus Fert & Ster 17 621 1966 d

Csapo A The Termination of Pregnancy by the Intra-Amniotic Injection of Hypertonic Saline (1966–1967 Year Book of Obstetrics & Gynecology) Ed J P Greenhill Year Book Medical Publishers

Friedman E Niswander K R Bayonet-Rivera N P and Sachtleben M R Relation of Prelabor Evaluation to Inducibility and the Course of Labor Obst & Gynec 28 495, 1966

Fuchs A R Oxytocin and the Onset of Labour in Rabbits J Endocrin. 30 217 1964

Gillespie E C Principles of Uterine Growth in Pregnancy Am J Obst. & Gyn 59 949 1950

Hendricks C H Comments on the Prevention of Prematurity PHYSIOLOGY OF PREMATURITY Trans. Fifth Conf ed. M Kowlessar Spons by Josiah Macy Jr Foundation, 1960

Kumar D and Barnes A C Studies in Human Myometrium During Pregnancy Article 6 Tissue Progesterone Profile of the Various Compartments in the Same Individual Am J Obst & Gyn 92 717 1965

C R Gulick F B Long, N K. Muschany and J B Martin for stimulating discussions and their cooperation in providing us with the study patients Our special thanks are due to research nurse Judy Gamblin and Carole English for their dedicated attention of the patients and Elise Gramss and Vivian Hertelendy for the analysis of the tracings

REFERENCES

Bengtsson L. Ph and *Csapo A. I* Oxytocin Response Withdrawal and Reinforcement of Defense Mechanism of the Human Uterus at Midpregnancy Am J Obst. & *Gyn 83* 1083 1962

Burnhill M S Daniels J and *Cohen J* Uterine Contractility During Labor Studied by Ultra-Amniotic Fluid Pressure Recordings Am. J Obst. & Gyn 83 561 1962 a

Burnhill M Gaines J and *Guttmacher A.* Concentrated Oxytocin Solution for Therapeutic Interruption of Midtrimester Pregnancy *Obst.* & Gynec. 20 94 1962 b

Caldeyro-Barcia R. Factors Controlling the Action of the Pregnant Human Uterus PHYSIOLOGY OF PREMATURITY Trans Fifth Conf ed. M. Kowlessar Spons by Josiah Macy Jr Foundation, 1950

Caldeyro-Barcia R Regulation of Myometrial Activity in Pregnancy MUSCLE, Proc. Symposium at the Faculty of Medicine Univ of Alberta 1964 Eds W M Paul E. E. Daniel C M. Kay & G Monckton, Pergamon Press

Cassmer O Hormone Production of the Isolated Human Placenta Studies on the Role of the Foetus in the Endocrine Function of the Placenta, Acta Endocrin. Suppl 45 1948

Coutinho E. M Hormone Induced Ionic Regulation of Labor Second International Congress of Endocrinology International Congress Series No 83 London 1964

Csapo A. The Asymmetrical Uterus and the Mechanism of Parturition PHYSIOLOGY OF PREMATURITY Trans Fifth Conf ed. M. Kowlessar Spons by Josiah Macy Jr Foundation 19C0

Csapo A. The Effects of Oxytocic Substances on the Excitability of the Uterus OXYTOCIN—AN INTERNATIONAL SYMPOSIUM Pergamon Press 1961 a

Csapo A. On Progesterone and the Defence Mechanism of Pregnancy Ciba Foundation Study Group No 9 1961 b

Csapo A. I Jaffin H Kerenyi T Lipman J and *Wood C* Volume and Activity of the Pregnant Human Uterus Am J Obst & Gyn. 85 619 1963 a

Csapo A. I Takeda H and *Wood C* Volume and Activity of the Parturient Rabbit Uterus Am. J Obst. & Gyn 85 813 1963 b

Csapo A. I and Takeda, H Electrical Activity of the Parturient Human Uterus, Nature 200 680 1963 c
Csapo A I. Jaffin H Kerenyi T de Mattos C. E. R. and Souza-Filho M. B Fetal Death in Utero Am. J Obst. & Gyn. 87 892, 1963 d
Csapo A I Extraovular Pressure—Its Diagnostic Value Am. J Obst. & Gyn 90 493 1964
Csapo A., The Intra-Uterine Control of Parturition Second International Congress of Endocrinology International Congress Series No. 83 London 1964 b
Csapo A The Placenta and the Initiation of Labor Nederlandsch Tijdschrift voor Verloskunde en Gynaecologie 65 229 1965 a
Csapo A. I. and Takeda H Effect of Progesterone on the Electric Activity and Intrauterine Pressure of Pregnant and Parturient Rabbits Am J Obst. & Gyn 91 221 1965 b
Csapo A Erdos T De Mattos C R. Gramss E and Moscowitz C. Stretch-Induced Uterine Growth Protein Synthesis and Function Natur 207 1378 1965
Csapo A. I and Pinto-Dantas C A The Cyclic Activity of the Non-Pregnant Human Uterus Fert & Ster 17 34 1966 a
Csapo A I Pinto-Dantas C A. and Kerenyi T Progesterone and Myometrial Activity in press from Fifth World Congress on Fertility and Sterility Stockholm, 1966 b
Csapo A I Kerenyi T De Souza-Filho M B Pinto-Dantas C. A. De Souza O Daree E and Ogata S. Control of the Lengths of the Menstrual Cycle and Gestation, Presented at Symposium of the Society for the Study of Fertility Cambridge 1966
Csapo A De Souza-Filho M B De Souza J C and De Souza O Effect of Massive Progestational Hormone Treatment on the Parturient Human Uterus Fert & Ster 17 621 1966 d
Csapo A The Termination of Pregnancy by the Intra-Amniotic Injection of Hypertonic Saline (1966–1967 Year Book of Obstetrics & Gynecology) Ed J P G reenhill Year Book Medical Publishers
Friedman E Niswander K R Bayonet-Rivera N P and Sachtleben M R Relation of Prelabor Evaluation to Inducibility and the Course of Labor Obst & Gynec 28 495 1966
Fuchs A R Oxytocin and the Onset of Labour in Rabbits J Endocrin. 30 21 1964
Gillespie E C Principles of Uterine Growth in Pregnancy Am J Obst. & Gyn 59 949 1950
Hendricks C H Comment on the Prevention of Prematurity PHYSIOLOGY OF PREMATURITY Trans. Fifth Conf ed. M Kowlessar Spons. by Josiah Macy Jr Foundation, 1960
Kumar D and Barnes A C Studies in Human Myometrium During Pregnancy Article 6 Tissue Progesterone Profile of the Various Compartments in the Same Individual Am J Obst & Gyn 92 717 1965

Kuriyama H Recent Studies on the Electrophysiology of the Uterus in Progesterone and the Defence Mechanism of Pregnancy Ciba Foundation Study Group No 9 1961 a

Kuriyama H & *Csapo A* A Study of the Parturient Uterus with Microelectrode Technique Endocrin. 68 1010 1961 b

MilUuall G Noall M W and *Allen W M* Progesterone Levels in the Rabbit Ovarian Vein Blood Throughout Pregnancy Endocrin. 69 504 1961

Page E. W Response of Human Pregnant Uterus to Pitocin Tannate in Oil, Proc. Soc. Exper Biol & Med. 52 195 1943

Porter D G and *Schofield B M* Intra-Uterine Pressure Changes During Pregnancy and Parturition in Rabbits J Endocrin. 36 291 1966

Reynolds S R. M PHYSIOLOGY OF THE UTERUS second ed. New York, Paul B Hoeber Inc 1949

Rhodes P The Volume of Liquor Amnii in Early Pregnancy J Obst. & Gyn. Brit Comm. 73 21 1966

Short R. V and *Eton B* Progesterone in the Peripheral Blood of Pregnant Women J Endocrin. 18 418 1959

Sureau C Étude de l activité électrique de l utérus au cours de la gestation et du travail Thèse Paris 1955

Theobald G W The Synthesis of Divergent Observations Concerning Oxytocin OXYTOCIN—AN INTERNATIONAL SYMPOSIUM, Pergamon Press 1961

Townsend S L Mackay E. V Shelton J G Krieger V I and *Campbell K. I* Induction of Labour in the Rh-Immunized Patient J Obst. & Gyn. Brit. Comm. 68 382, 1961

Wiest W Estimation of Progesterone in Biological Tissues and Fluids from Pregnant Women by Double Isotope Derivative Assay Steroids 10:3 279 1967

Woolever C A. Progesterone and Progesterone Therapy in Pregnancy Clinical Obstetrics & Gynecology ed. W F Manley Hoeber Medical Division 1965

Zander J Gestagens in Human Pregnancy from Recent Progress in the Endocrinology of Reproduction Academi Press New York and London, ed. C W Lloyd, 1959

Zander J Die Behandlung der bedrohten Schwangerschaft, Archi fur Gynäkologie 1967 (i press)

Received on May 16 1967

Nilsson, Ingrid and Bengtsson, Lars Ph. Acta obst. et gynec. scandinav. 47: 223, 1968
From the Department of Obstetrics and Gynaecology (Prof. Alf Sjövall)
University Hospital of Lund, Sweden

GAS CHROMATOGRAPHIC DETERMINATION OF URINARY OESTRIOL IN INTRA UTERINE FOETAL DEATH

BY

INGRID NILSSON AND LARS PH. BENGTSSON

Estimation of the urinary oestriol excretion is of clinical value in pregnancy to study the condition of the foeto-placental unit, and in the non-pregnant state to check hypophyseal-gonadal function. During the normal menstrual cycle oestriol is excreted in small amounts 10–100 μg/24 h. (*Brown* 1955 a *Bauld* 1956 *Preedy* and *Aitken* 1961) During normal pregnancy oestriol excretion increases considerably up to 25 mg/24 h at term (*Brown* 1956 *Ittrich* and *Igel* 1959 *Frandsen* 1963 *Beling*, 1963 *Schwers* 1965) After foetal death urinary oestriol rapidly falls (*Zondek* 1954 *ten Berge* 1958 and *Cassmer* 1959) A lesser decrease may occur before foetal death, when the life of the foetus is endangered. (*Taylor et al.* 1955 *Greene* and *Touchstone* 1963 For other references see *ten Berge* 1965)

In the non-pregnant state determination of the small amounts of urinary oestriol necessitates extensive purification of the urine with laborious and time-consuming methods. (*Brown* 1955 b *Bauld* 1956 *Preedy* and *Aitken* 1961 and *Barlow* 1963.) For clinical evaluation of the condition of the foetus, on the other hand much simpler and more rapid methods give sufficient information, and a number of such methods have been published (*Ittrich* 1960 *Frandsen* 1963 *Fishman* and *Brown* 1962 *Beling*, 1963 *Wotiz* and *Chattoraj* 1964)

Previous studies in our department (*Bengtsson* 1962 and 1965 *Bengtsson* and *Forsgren* 1966) showed that after foetal death oestriol excretion may ultimately approach non-pregnant values In our continued study of the excretion of oestriol in missed

abortion we needed a method more sensitive and more accurate than those used in normal pregnancy but less complicated and less time-consuming than those needed in non pregnant women. The following method was therefore developed.

Material and method

All reagents were of analytical grade

Solvents were purified as recommended by *Bush* (1961) and *Neher* (1964)

Enzymatic hydrolysis was carried out with Helix Pomatia extract (100 000 Fishman units of β-glucuronidase and 100 000 Roy units of sulphatase per ml juice) Gas chromatography was performed by a Perkin Elmer 800 apparatus. The column was a 2 m, 2.2 mm i d stainless steel tube filled with 2.4 % XE-60 The carrier was Chromosorb W 80–100 mesh, treated with trimethyl chlorosilane and hexamethyldisilazane.

Operation conditions The column was kept at 250 C and the injector at about 300 C The nitrogen flow was between 70 and 80 ml/min. measured at the outlet of the column. The air pressure was 2.0 kg/cm^2 and the hydrogen pressure was kept at about 1.35 kg/cm^2 as measured with an extra manometer between the gas tube and the inlet of the apparatus These gas pressures corresponded to the manufacturer's recommendation of gas flows of 300–400 ml/min and 30 ml/min. respectively

The curve area was calculated by multiplying peak height and width at mid height Standard curves with solutions of pure oestriol triacetate were made daily before analysis

Aanalytical procedure

The 24 hours collection of urine was stored in a refrigerator without preservative If not analyzed within a week it was kept deep frozen at –20 C

The method in detail

1 50 % $(NH_4)_2SO_4$ (w/v) is dissolved in one fifth of the 24 hours collection of urine Extraction with 3×1 volumes ether-ethanol 3 1

2. The ether-ethanol is evaporated and the residue dissolved in 100 ml acetate buffer 1 0 M pH 4 8
3. Incubation for 90 hours at 37 C with 50.000 units of β-glucuronidase and 50 000 units of sulphatase.
4. Extraction with ether-ethanol 4 1 3×100 ml.
5. The ether extract is washed with 30 ml 8 % $NaHCO_3$.
6. The extract is shaken with 15.0 ml 2.0 N NaOH. Then 60 ml 8 % NaHCO is added and the extract is shaken with the resulting buffer
7. The extract is washed with 20 ml 8 % $NaHCO_3$ and then with 10 ml distilled water
8. Reextraction with ether of all the water phases from 5–7 above. The combined ether extracts are evaporated.
9. The residue is dissolved in 2 ml ether and transferred to a separating funnel with $3\times\frac{50}{3}$ ml toluene. The toluene is extracted with 3×20 ml 1 0 N NaOH.
10. Solid $NaHCO_3$ is added to pH 9.5–10.0
11. Reextraction with 3×50 ml ether
12. The ether is evaporated and the residue dissolved in 1 ml absolute ethanol.
13. The extract is transferred to a separating funnel with $3\times\frac{50}{3}$ ml benzene—light petroleum (bpt 40°–60° C)
14. The oestriol fraction is extracted with 2×25 ml distilled water
15. Extraction of the water extract with ether 50+25+25 ml. E aporation of the ether
16. The dry residue is acetylated with 0.5 ml acetic anhydride in 0 1 ml pyridine at 70 C for one hour or at room temperature over night. It is then evaporated.
17. The sample is dissolved in ethylene dichloride and analyzed with GLC For analytical scheme see Fig. 1

Extraction of the conjugates according to *Edwards Kellie* and *Wade* (1953) was made to reduce and standardize the sample olume and to remove some interfering pigments.

The distribution between toluene and sodium hydroxide was originally described by *Diczfalusy* and *Lindkvist* (1956) to re-

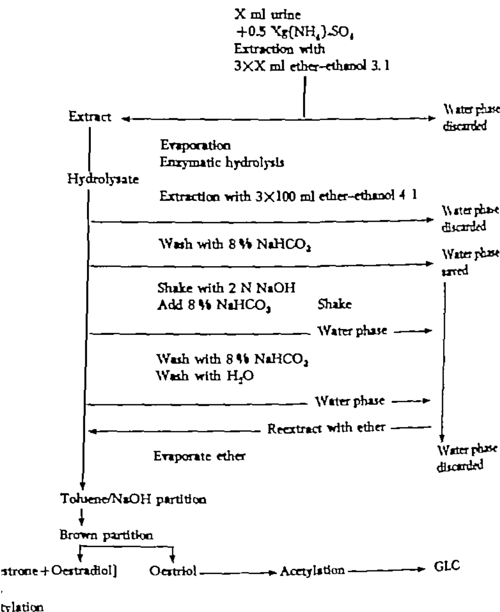


Fig. 1. Analytical scheme for gas chromatographic determination of urinary oestriol

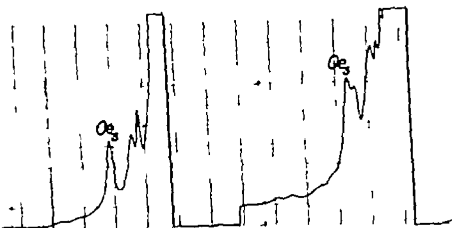


Fig. 2. Gas chromatogram of urinary oestriol one month after intra-uterine foetal death. Purification with (left) and without (right) partition between toluene and sodium hydroxide. Oe_3=oestriol peak.

move fatty material from blood samples. The effect of the procedure on urine samples is illustrated by Fig. 2.

Identification of the oestriol peak

Extracts from male urine showed no peak with the same retention time as pure oestriol triacetate. Extracts from male urine with added oestriol and also urinary extracts from pregnant women and from cases of intrauterine foetal death showed a single peak with the same retention time as pure oestriol triacetate.

The precision of the method was tested by recovery experiments and duplicate determinations. Recovery experiments were performed with free oestriol added to the urine as no conjugates were by then commercially available. This procedure is generally accepted although the results are approximated. The recovery is in the range of other methods and varies in different experiments (Table I). Thus the recovery rate must be checked in each series of determinations. As expected the recovery falls with the oestriol content of the samples.

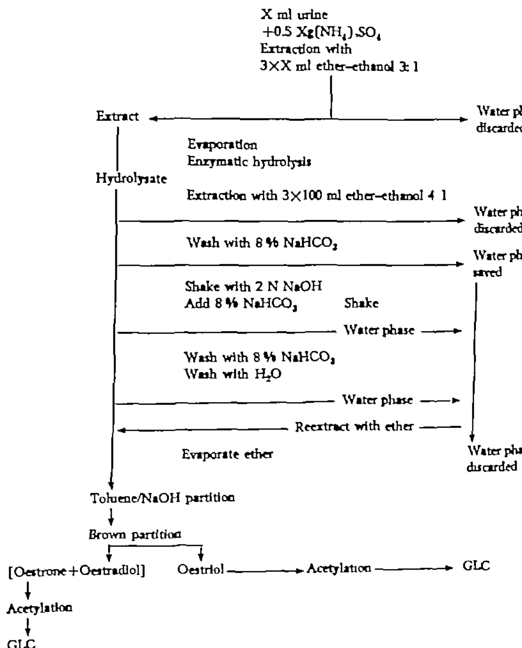


Fig 1 Analytical scheme for gas chromatographic determination of urinary oestriol

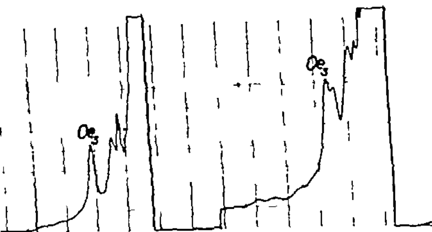


Fig. 2. Gas chromatogram of urinary oestriol one month after intra-uterine foetal death. Purification with (left) and without (right) partition between toluene and sodium hydroxide. Oe_3 = oestriol peak.

move fatty material from blood samples. The effect of the procedure on urine samples is illustrated by Fig. 2.

Identification of the oestriol peak

Extracts from male urine showed no peak with the same retention time as pure oestriol triacetate. Extracts from male urine with added oestriol and also urinary extracts from pregnant women and from cases of intrauterine foetal death showed a single peak with the same retention time as pure oestriol triacetate.

The precision of the method was tested by recovery experiments and duplicate determinations. Recovery experiments were performed with free oestriol added to the urine as no conjugates were by then commercially available. This procedure is generally accepted although the results are approximated. The recovery is in the range of other methods and varies in different experiments (Table I). Thus the recovery rate must be checked in each series of determinations. As expected the recovery falls with the oestriol content of the samples.

Table I. *Recovery of Added Oestriol*

μg Oestriol Added to 200 ml Male Urine	μg Oestriol Recovered	Mean	% Deviation	% Recovery
125	96 100	98 ± 2.0	± 2.0	78.5
125	94 90 96	93.5 ± 3.5	± 3 7	74 0
50	34 36 38	36 0 ± 2.0	± 5.5	72.0

Table II *Deviation from the Mean Value in Duplicate Determinations*

μg Oestriol per 24 Hours	Mean	% Deviation
268 285	276 ± 9	± 3.3
400 360	380 ± 20	± 5.2
487 526	506 ± 20	± 4.0
4380 4210	4295 ± 85	± 2.0

The duplicate determinations (Table II) show a satisfactorily low deviation. The lower limit of the method is 100 μg/24 h urine.

Results

Oestriol excretion in normal pregnancy

The method was tested in six patients with normal pregnancies from the 14th to the 33rd week of gestation. (See example in Fig 3) As is evident from Fig 4 the values obtained are well within the ranges found by other methods

Table III. *Urinary Excretion of Oestriol in Intra-uterine Foetal Death*

Case	Month of Gestation	Foetus Dead for	µg Oestriol per 24 h.
E.M.	VIII	1 week	510
Eva M	VIII	10 days	430
G H	IX	2 weeks	280
R G	VIII	1 month	380
G O	VI	1 month	345
A.M H.	VII	1 month	300
G N	VII	1 month	220
E C	VIII	1 2 months	250
E M.	V	1 month	125
I B.A.	VI	1 month	~90
B N	VII	1 month	~60
M H	V	2 months	~20

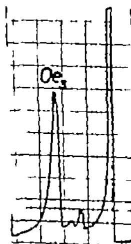


Fig. 3 Gas chromatogram of urinary oestriol in the 27th week of normal pregnancy

Oestriol excretion after intra-uterine foetal death

The urinary excretion of oestriol was determined in 12 cases of intra-uterine foetal death (Table III and Fig. 5) The estimated period between foetal death and oestriol determination varied

Table I. *Recovery of Added Oestriol*

μg Oestriol Added to 200 ml Male Urine	μg Oestriol Recovered	Mean	% Deviation	% Recovery
125	96 100	98 ± 2.0	± 2.0	78.5
125	94 90 96	93.5 ± 3.5	± 3.7	74.0
50	34 36 38	36.0 ± 2.0	± 5.5	72.0

Table II *Deviation from the Mean Value in Duplicate Determinations*

μg Oestriol per 24 Hours	Mean	% Deviation
268 285	276 ± 9	± 3.3
400 360	380 ± 20	± 5.2
487 526	506 ± 20	± 4.0
4380 4210	4295 ± 85	± 2.0

The duplicate determinations (Table II) show a satisfactorily low deviation. The lower limit of the method is 100 μg/24 h urine.

Results

Oestriol excretion in normal pregnancy

The method was tested in six patients with normal pregnancies from the 14th to the 33rd week of gestation (See example in Fig 3) As is evident from Fig 4 the values obtained are well within the ranges found by other methods

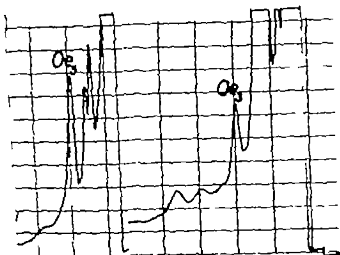


Fig. 5. Gas chromatograms of urinary oestriol. Left: One month after foetal death. The peak corresponds to 300 µg/24 h. Right: More than one month after foetal death. The peak corresponds to 125 µg/24 h.

Discussion

It is well known that acid hydrolysis of urine destroys some steroids and gives varying recovery rates. In this study, therefore, enzymatic hydrolysis was performed. This also enables the determination of not only oestrogens but also other steroids in the same hydrolysate.

SE 30 has been the column used most commonly for oestrogen determination. After the described purification procedures, however, we found that oestriol appears in the gas chromatogram with SE 30 columns together with an impurity. XE 60, on the other hand, gave only one peak with the same retention time as pure oestriol even when the chromatogram was run with a slow temperature programme.

The method described also permits determination of *oestrone* from the same extract (after Brown partition) by means of a SE 30 column at a temperature of about 230° C. Values between 60 and 115 µg were found in three cases where the foetus had been dead for 2–4 weeks. Other experimental conditions were the same as described for oestriol.

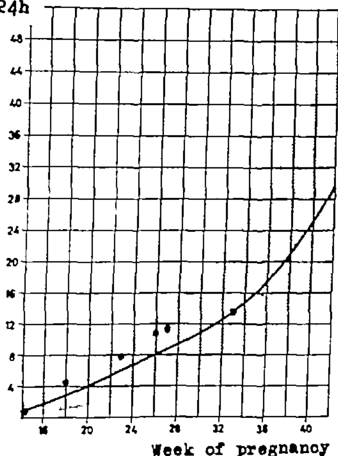


Fig 4 Urinary oestriol in 6 normal pregnancies The normal range is from Frandsen et al (1962)

from one week to two months The length of gestation at foetal death varied between five and nine months. The results presented in Table III indicate that after the rapid fall following foetal death there is a slow but steady decrease in oestriol excretion. Non pregnant values (*i e* below 100 μg/24 h) are finally reached, but not until more than one month after foetal death. The values obtained by this method are well in accordance with those found in our earlier investigations on intra uterine foetal death (*Bengtsson* 1962 and 1965 *Bengtsson* and *Forsgren* 1966)

SUMMARY

A purification scheme is presented for gas chromatographic determination of urinary oestriol down to a level of 100 μg/24 h. The purification involves extraction of the conjugates, enzymatic hydrolysis, extraction of the phenolic fraction, separation of the oestriol fraction and gas chromatographic determination of oestriol in the acetylated extract. The method was tested on urine from some normal pregnant women and applied in twelve cases of intra-uterine foetal death. After the abrupt fall in urinary oestriol excretion which is known to rapidly follow intra-uterine death, a further slow but continuous decline was observed. Non-pregnant values were found in patients with intra-uterine death of one to two months duration.

Acknowledgement

This study was supported by the Ford Foundation.

REFERENCES

Barlow J J Anal. Chem 6 435, 1963
Bauld, W S Biochem J 63 488 1956
Belling, C G Acta Endocrinol (Kbh) Suppl 79 1963
Bengtsson L Ph Lancet I 339 1962
In Advances in Oxytocin, p 87 Pergamon Press 1963
Bengtsson L Ph and Forsgren B Acta obst et gynec. scandinav 45 155 1966
ten Berge B S Geburtsh Frauenh 18 323 1958
Pregnancy Chemistry and Management Charles C. Thomas Publ Springfield Illinois U S A 1963 Ed ten Berge B. S.
Brown J B Lancet I 320 1955
Biochem J 60 185 1955 b
Lancet I 704 1956
Bush I E In The Chromatography of Steroids Pergamon Press Ltd Oxford 1961
Cassmer O Acta Endocrinol (Kbh) Suppl 45 1959
Diczfalusy E and Lindkvist P Acta Endocrinol (Kbh) 22 203 1956
Edwards R W H Kellie A E and Wade A P Mem Soc. Endocrinol. London Dobson 2 53 1953
Fishman J and Brown J B J of Chromatography 8 21 1962
Frandsen V A Pedersen J and Stakemann G Acta Endocrinol (Kbh) 40 400 1962

Oestradiol appeared together with some impurities and could not be determined without additional procedures.

This investigation has shown that when reliable results are wanted from low titre urine even the aid of gas chromatography *necessitates careful purification* of the urine. However using the present scheme with GLC as the final step to determine oestriol, purification by means of thin layer or column chromatography could be excluded. This we regard as a valuable advantage because we could avoid preparatory steps which include hazardous *standardisation of columns or plates*

The abrupt fall in urinary oestriol immediately after intra uterine foetal death is followed by a very slow fall over several weeks The first, rapid fall indicates that the normally functioning foeto-placental unit is responsible for the major part of oestriol *produced during pregnancy The subsequent slow fall indicates* that the placenta *alone* is also capable of some oestrogen production. This has also been demonstrated in the very rare cases of a full term extra-uterine pregnancy in which the living foetus is removed and the placenta left intact in the abdominal cavity (*Michie* 1966) An ovarian source of oestrogen as is observed in hydatidiform mole and chorionepithelioma (*Frandsen* and *Stakemann* 1964) is less probable as the gonadotrophic stimulation is much lower in association with missed abortion.

The second, slow fall in oestriol excretion may theoretically be explained in another way oestrogen stored in tissue depots is released rather slowly It seems improbable however that the depots continue to release oestrogen for several weeks.

The oestriol determinations in our 12 cases of intra uterine foetal death support our earlier findings that in such cases oestriol excretion may eventually reach non pregnant values It should be noted that we have covered the range from one week to two months between foetal death and oestriol determination.

Schwers (1965) has presented oestriol excretion values in a series of intra uterine foetal deaths which differ from ours. The difference is probably due to the fact that in most of Schwers cases the time between foetal death and oestriol determination was only a few days in the rest the time was unknown.

Moawad, Atef H and Bengtsson, Lars Ph. Acta obst. et gynec. scandinav 47 725, 1968
From the Department of Obstetrics and Gynaecology (Prof Alf Sjövall)
University Hospital of Lund Sweden

IN VIVO STUDIES OF THE MOTILITY PATTERNS OF THE NON PREGNANT HUMAN UTERUS

II. The effect of Oestrogen on Myometrial Activity

BY

ATEF H. MOAWAD[1] AND LARS PH. BENGTSSON

The myometrial activity of the human uterus *in vivo* in the various phases of the menstrual cycle has been studied by a series of authors, among others *Schultze* (1931) *Moir* (1934) *Henry* and *Browne* (1943) *Posse* (1958) *Csapo* and *Pinto-Dantas* (1966) *Hendricks* (1966) *Moawad* and *Bengtsson* (1967) and *Cibils* (1967) Most authors agree that certain activity patterns follow each other successively and repeat themselves periodically during the menstrual cycles It is appealing to assume that each of these characteristic patterns is related to one or both ovarian hormones Therefore, we have tried to study uterine activity under conditions where one hormone was dominant. This presentation deals with oestrogen as the dominant hormone

Method and Material

The uterine activity was recorded by using the open end catheter technique (*Hendricks* 1964) Details of this technique as used by us are described in a preceding report (*Moawad* and *Bengtsson* 1967) The patients were recorded from twice weekly to daily each time for at least two hours.

Three conditions have been studied, all of them characterized by pronounced oestrogen dominance i.e. oestrogen without or with negligible amounts of progesterone

[1] Personal grants from the Bergh Foundation.

Frandsen V A. The excretion of oestriol in normal pregnancy Ed. Munksgaard, Copenhagen 1963
Frandsen V A. and *Stakemann G* Acta Endocrinol (Kbh) *Suppl 90* 81 1964
Greene J W and *Touchstone J C.* Am J Obst. & Gynec. *85* 1 1963
Ittrich G Acta Endocrinol. (Kbh) *35* 34 1960
Ittrich G and *Igel H* Zentralbl Gynäk. *81* 255 1959
Michie E A. Acta Endocrinol. (Kbh) *51* 535 1966
Neher R. In. Steroid Chromatography Elsevier Publ Co. Amsterdam 1964
Preedy J R. K. and *Aitken E. H* J Biol Chem. *236* 1300 1961
Schuers J Les oestrogènes au cours de la seconde moitié de la grossesse. Edition Arscia S A. Bruxelles 1955
Taylor E. S Bruns P D Anker R. M. and *Drose V E.* Am. J Obst. & Gynec. *70* 894 1955
Wotl H and *Chattoraj S* Anal. Chem *36* 1466 1964
Zondek B Recent Progress of Hormonal Research *10* 395 1954 Academic Press Inc New York

Received on June 1 1967

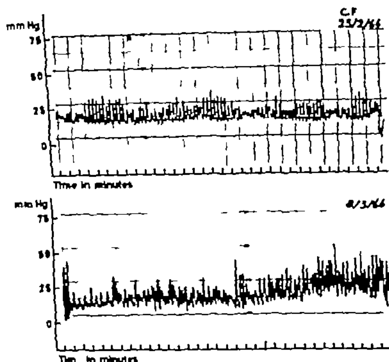


Fig 1 Intra-uterine recordings taken 2 weeks apart in case of Stein-Leventhal syndrome

which are typical of the late secretory phase and which appear earlier when an IUD is present (Bengtsson and Moawad, 1967)

Figure 3 shows the activity pattern from the castrated woman before hormonal treatment irregular contractions with very low amplitude By the fourth day of oestrogen treatment the myometrial activity changed into a typical proliferative phase pattern, which was then maintained throughout the period of treatment. Fig 4 shows the activity on the 26th day of oestrogen treatment. Again the lack of prelabour and labour-like activity was evident throughout the whole period of therapy Also withdrawal of the hormone did not bring out the characteristic menstrual pattern (similar to active labour) that is usually seen in normal ovulatory cycles Fig 5 shows the tracing taken 36 hours after the admini-

1 A 24-year old woman with past history of primary infertility hirsutism and irregular menstrual cycles. She was treated surgically by wedge resection of both ovaries the histology of which was compatible with polycystic disease of the Stein-Leventhal type. She ovulated spontaneously following the therapy and had a normal pregnancy and delivery of a full term normal infant. She then developed post partum amenorrhea and at the time of the recording seven months had passed without any sign of menstrual flow She had a monophasic temperature chart, an oestrogenic vaginal smear and negligible pregnanediol excretion in the urine.
2. Two cases with spontaneous anovulatory menstrual cycles showing good oestrogen effects. In both cases the diagnosis was based on basal body temperature serial vaginal smears and negligible pregnanediol excretion. One of these subjects had an intra uterine contraceptive device (Lippes loop D) throughout the study
3 One case of oestrogen treatment six years after castration. Ethinyloestradiol was given in doses intended to imitate the physiological amounts of oestrogen endogenously produced during a normal menstrual cycle starting from 10/μg ethinyloestradiol daily with daily increases of 10/μg up to 100/μg per day which dose was maintained for six days. Thereafter daily decreases by 10/μg until 20/μg per day was reached on the 23rd day This dose was maintained for 3 days and then the therapy was withdrawn.

Results

Figure 1 shows two tracings taken two weeks apart from the woman with Stein Leventhal syndrome Both records show the activity pattern typical for the proliferative phase of ovulatory cycles.

The same activity pattern was obtained in the two cases of anovulatory cycles It should be observed that even in the presence of a Lippes loop (Fig 2) the activity pattern throughout the cycle was the same as in the other cases studied Thus there is no evolution of the slow high amplitude contractions

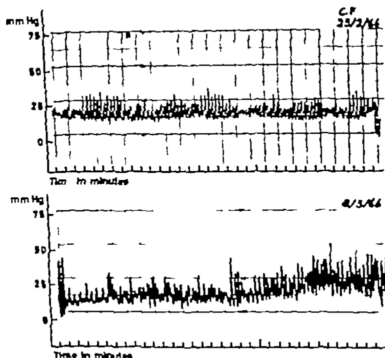


Fig 1 Intra-uterine recordings taken 2 weeks apart in case of Stein-Leventhal syndrome

which are typical of the late secretory phase and which appear earlier when an IUD is present (Bengtsson and Moawad, 1967)

Figure 3 shows the activity pattern from the castrated woman before hormonal treatment Irregular contractions with very low amplitude By the fourth day of oestrogen treatment the myometrial activity changed into a typical proliferative phase pattern, which was then maintained throughout the period of treatment. Fig 4 shows the activity on the 26th day of oestrogen treatment. Again the lack of prelabour and labour-like activity was evident throughout the whole period of therapy Also withdrawal of the hormone did not bring out the characteristic menstrual pattern (similar to active labour) that is usually seen in normal ovulatory cycles Fig. 5 shows the tracing taken 36 hours after the admini-

1 A 24-year old woman with past history of primary infertility hirsutism and irregular menstrual cycles. She was treated surgically by wedge resection of both ovaries the histology of which was compatible with polycystic disease of the Stein-Leventhal type She ovulated spontaneously following the therapy and had a normal pregnancy and delivery of a full term normal infant. She then developed post partum amenor rhea and at the time of the recording seven months had pas sed without any sign of menstrual flow She had a monophasic temperature chart, an oestrogenic vaginal smear and negligible pregnanediol excretion in the urine
2. Two cases with spontaneous anovulatory menstrual cycles showing good oestrogen effects In both cases the diagnosis was based on basal body temperature serial vaginal smears and negligible pregnanediol excretion. One of these subjects had an intra uterine contraceptive device (Lippes loop D) throughout the study
3 One case of oestrogen treatment six years after castration. Ethinyloestradiol was given in doses intended to imitate the physiological amounts of oestrogen endogenously produced during a normal menstrual cycle starting from 10/μg ethinyloestradiol daily with daily increases of 10/μg up to 100/μg per day which dose was maintained for six days. Thereafter daily decreases by 10/μg until 20/μg per day was reached on the 23rd day This dose was maintained for 3 days and then the therapy was withdrawn.

Results

Figure 1 shows two tracings taken two weeks apart from the woman with Stein Leventhal syndrome Both records show the activity pattern typical for the proliferative phase of ovulatory cycles

The same activity pattern was obtained in the two cases of anovulatory cycles It should be observed that even in the presence of a Lippes loop (Fig 2) the activity pattern through out the cycle was the same as in the other cases studied Thus there is no evolution of the slow high amplitude contractions

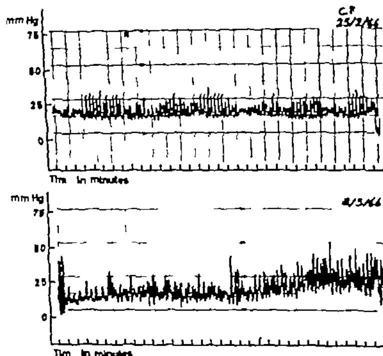


Fig 1 Intra-uterine recordings taken 2 weeks apart in case of Stein-Leventhal syndrome.

which are typical of the late secretory phase and which appear earlier when an IUD is present (Bengtsson and Moawad, 1967)

Figure 3 shows the activity pattern from the castrated woman before hormonal treatment. Irregular contractions with very low amplitude By the fourth day of oestrogen treatment the myometrial activity changed into a typical proliferative phase pattern, which was then maintained throughout the period of treatment. Fig. 4 shows the activity on the 26th day of oestrogen treatment. Again the lack of prelabour and labour-like activity was evident throughout the whole period of therapy Also, withdrawal of the hormone did not bring out the characteristic menstrual pattern (similar to active labour) that is usually seen in normal ovulatory cycles Fig. 5 shows the tracing taken 36 hours after the admini

1 A 24-year old woman with past history of primary infertility hirsutism and irregular menstrual cycles She was treated surgically by wedge resection of both ovaries the histology of which was compatible with polycystic disease of the Stein-Leventhal type She ovulated spontaneously following the therapy and had a normal pregnancy and delivery of a full term normal infant. She then developed post partum amenor rhea and at the time of the recording seven months had pas sed without any sign of menstrual flow She had a monophasic temperature chart, an oestrogenic vaginal smear and negligible pregnanediol excretion in the urine
2. Two cases with spontaneous anovulatory menstrual cycles showing good oestrogen effects In both cases the diagnosis was based on basal body temperature serial vaginal smears and negligible pregnanediol excretion. One of these subjects had an intra uterine contraceptive device (Lippes loop D) throughout the study
3 One case of oestrogen treatment six years after castration. Ethinyloestradiol was given in doses intended to imitate the physiological amounts of oestrogen endogenously produced during a normal menstrual cycle starting from 10/μg ethinyloestradiol daily with daily increases of 10/μg up to 100/μg per day which dose was maintained for six days. Thereafter daily decreases by 10/μg until 20/μg per day was reached on the 23rd day This dose was maintained for 3 days and then the therapy was withdrawn

Results

Figure 1 shows two tracings taken two weeks apart from the woman with Stein Leventhal syndrome Both records show the activity pattern typical for the proliferative phase of ovulatory cycles

The same activity pattern was obtained in the two cases of anovulatory cycles It should be observed that even in the presence of a Lippes loop (Fig 2) the activity pattern through out the cycle was the same as in the other cases studied Thus there is no evolution of the slow high amplitude contractions

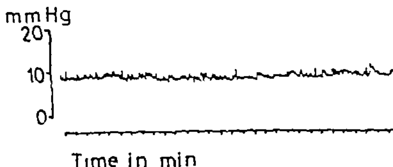


Fig. 3. Intra-uterine recording from a woman 6 years after castration.

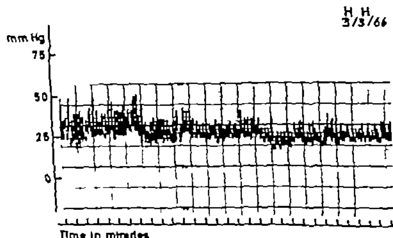


Fig. 4. Intra-uterine recording from the same woman as in Fig. 3 after 26 days oestrogen treatment.

Discussion

In all the three conditions characterized by uterine stimulation with oestrogen alone the myometrial activity pattern was closely similar to that in the proliferative phase of normal menstrual cycles as described in our previous publication (*Moawad* and Bengtsson 1967) namely frequent contractions (2–4 per min.)

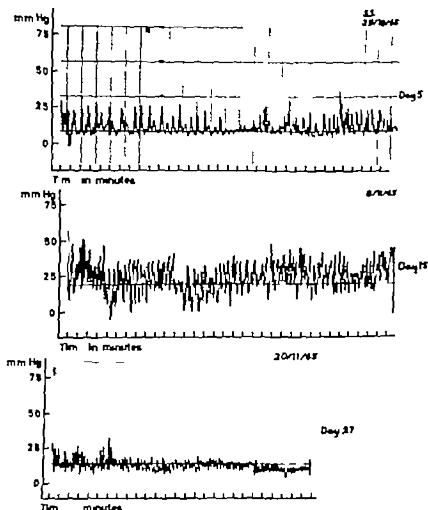


Fig 2. Intra-uterine recordings on days 5 15 and 27 in an anovulatory cycle of 28 days

stration of the last dose of oestrogen and which coincided with the withdrawal bleeding Although there is a change in frequency and duration of the contractions they are different from the labour like contractions seen at the onset of normal menstrual flow

showed marked differences in myometrial activity between the proliferative and the luteal phases of the cycle in response to various stimuli. *Hendricks* (1966) reported that the uterus is more sensitive to injection of ADH in the secretory premenstrual and menstrual phases than during the proliferative phase. *Cibils* (1967) reported strong uterine contractions immediately after awakening in the secretory phase and their absence in the proliferative phase. He mentions this response is progesterone (or progestogen) dependent because it is absent in anovulatory cycles and menstruation and consistently obtained in progestogen treated patients. Similar results were reported in response to emotional stimuli, as early as 1939 by *Robertson*. Moreover presacral nerve electrical stimulation was shown to cause stronger myometrial response in the luteal phase of the cycle (*Caldeyro-Barcia* and *Alvarez* 1954).

This encourages us to put forward the hypothesis that the non-pregnant human uterus under the effect of physiological amounts of progesterone attains a degree of efficiency that enables it to respond more vigorously to various stimuli. Although this effect is hormone dependent, it might be still mediated through a neural mechanism.

SUMMARY

By means of open end catheters the myometrial activity *in vivo* has been studied in conditions of oestrogen dominance anovulatory cycles amenorrhoea due to the Stein-Leventhal syndrome and after treatment with ethinyloestradiol in a castrated woman. The activity pattern in all these conditions was closely similar to that in the proliferative phase of normal ovulatory cycles. Pre-labour and labour-like activity did not appear either during or after administration of exogenous oestrogen. The presence of an IUD did not affect the anovulatory pattern.

Acknowledgement

This investigation was supported by the Ford Foundation.

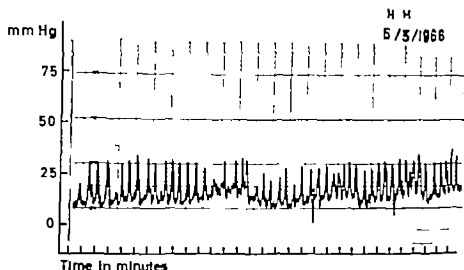


Fig 5 Intra-uterine recording from the same woman as in Figs 3 and 4 at the onset of bleeding after withdrawal of oestrogen treatment.

of low amplitude (up to about 15 mm Hg) short duration and occasional variation of regularity and resting pressure All cases completely lacked the slower contractions of higher amplitude which in ovulatory cycles appear a few days after ovulation.

In one of our previous reports we have shown that a prelabour like pattern can be brought about by the administration of progestational agents (*Moawad* and *Bengtsson* 1966) This is complementary to the results of this report.

This is also in agreement with *Garrett* s work (1959) in which he has shown the absence of the so-called B waves in spontaneously anovulatory cycles.

The lack of a change in the myometrial contractility despite the presence of an IUD in a uterus stimulated only by oestrogen is also in agreement with previous findings (*Bengtsson* and *Moawad* 1967) in which we have shown that the change in activity does not occur prior to the 19th day in normal ovulatory cycles. It seems therefore that the uterus does not react to mechanical stimuli with the same vigour prior to the addition of normal amounts of progestational hormones. This is not surprising if one goes back to the work of previous investigators who

showed marked differences in myometrial activity between the proliferative and the luteal phases of the cycle in response to various stimuli. *Hendricks* (1966) reported that the uterus is more sensitive to injection of ADH in the secretory premenstrual and menstrual phases than during the proliferative phase. Cibils (1967) reported strong uterine contractions immediately after awakening in the secretory phase and their absence in the proliferative phase. He mentions this response is progesterone (or progestogen) dependent because it is absent in anovulatory cycles and menstruation and consistently obtained in progestogen treated patients. Similar results were reported in response to emotional stimuli, as early as 1939 by *Robertson*. Moreover presacral nerve electrical stimulation was shown to cause stronger myometrial response in the luteal phase of the cycle (*Caldeyro-Barcia* and *Alvarez* 1954).

This encourages us to put forward the hypothesis that the non-pregnant human uterus under the effect of physiological amounts of progesterone attains a degree of efficiency that enables it to respond more vigorously to various stimuli. Although this effect is hormone dependent, it might be still mediated through a neural mechanism.

SUMMARY

By means of open end catheters the myometrial activity *in vivo* has been studied in conditions of oestrogen dominance: anovulatory cycles, amenorrhoea due to the Stein-Leventhal syndrome and after treatment with ethinyloestradiol in a castrated woman. The activity pattern in all these conditions was closely similar to that in the proliferative phase of normal ovulatory cycles. Pre-labour and labour-like activity did not appear either during or after administration of exogenous oestrogen. The presence of an IUD did not affect the anovulatory pattern.

Acknowledgement

This investigation was supported by the Ford Foundation.

REFERENCES

Bengtsson L. Ph. Carter A. and *Moawad A. H* Acta endocrinol. suppl. *119* 164 1967
Bengtsson L Ph. and *Moawad A. H* Am. J Obst. & Gynec. *98* 951 1967
Caldeyro-Barcia R. and *Alvarez H* J Appl Physiol *6* 556 1954
Cibils L. A. Obst. & Gynec. *30* 441 1967
Csapo A. I and *Pinto-Dantas C. R.* Fertil & Steril. *17* 34 1966
Garrett W J J Obst. & Gynec. Brit. Emp. *66* 602, 1959
Hendricks C. H J Obst. & Gynec. Brit. Cwlth *71* 712, 1964
– Am. J Obst. & Gynec. *96* 824 1966
Henry J S and *Browne I S L.* Am. J Obst. & Gynec. *45* 927 1943
Moawad A. H and *Bengtsson L. Ph.* Excerpta Med. Int. Congr Ser III 695 1966
– Am. J Obst. & Gynec. *98* 1057 1967
Moir C Edinburgh M.J *41* (Trans Edinb Obst. Soc. p. 93) 1934
Posse N Acta obst. et gynec. scandinav *37* Suppl. 2, 1958
Robertson E. H J Obst. & Gynec. Brit. Emp *46* 741 1939
Schultze G K. F Zentralbl. Gynäk. *55* 3042, 1931

Received on May 20 1967

A. H Moawad s present address:

Dept. of Obst & Gyn. and Pharmacology
University of Alberta
EDMONTON Canada

Hæger Knut, Acta obst et gynec scandinav 47 223, 1968
From the Department of Surgery Section for Vascular Surgery (*Prof Helge B Wulff*) Malmö General Hospital Malmö Sweden

THE TREATMENT OF VARICOSE VEINS IN PREGNANCY BY RADICAL OPERATION OR CONSERVATIVELY

BY

KNUT HÆGER

"Why should women suffer for months with a condition which is readily amenable to a safe and simple form of treatment?" (*W G Fegan*)
"The old bugaboo which deprives the pregnant woman of necessary surgery because of fear of initiating premature labor has been fairly well eliminated. (*F W Quattelbaum and Jane F Hodgson*)

The old bugaboo referred to in the quotation above was certainly true for the treatment of varicose veins in pregnancy until only some 15 years ago. *Quattelbaum* and *Hodgson* (1952) were not able to trace a single paper in which high ligation and stripping had been performed in all cases in which radical therapy for varicose veins in pregnancy was considered to be indicated.

However there had earlier been some reports giving evidence that, in limited series, surgery had been successful. In 1949 *Hamilton et al* *Peyton* and *Loop* and *Dodd* respectively reported good results of high ligation and stripping during pregnancy. *Dodd* concluded that treatment of troublesome varicose veins in pregnancy was permissible and justifiable. In 1945 *McPheeters* reported a series of patients treated with sapheno-femoral ligature and retrograde injection. Later however he re-examined his patients and abandoned this procedure since he found it "100 per cent unsuccessful" (*McPheeters* 1949). In spite of this statement

REFERENCES

Bengtsson L Ph Carter A. and *Moawad A. H* Acta endocrinol. suppl. *119* 164 1967
Bengtsson L Ph. and *Moawad A. H* Am J Obst. & Gynec. *98* 951 1967
Caldeyro-Barcia R. and *Alvarez H* J Appl Physiol *6* 556 1954
Cibils L A. Obst. & Gynec. *30* 441 1967
Csapo A. I and *Pinto-Dantas C R.* Fertil. & Steril *17* 34 1966
Garrett W J J Obst. & Gynec. Brit. Emp *66* 602, 1959
Hendricks C. H J Obst. & Gynec. Brit. Cwlth. *71* 712, 1964
– Am. J Obst. & Gynec. *96* 824 1966
Henry J S and *Browne I S L* Am. J Obst. & Gynec. *45* 927 1943
Moawad A. H and *Bengtsson L. Ph* Excerpta Med. Int. Congr Ser III 695 1966
– Am. J Obst. & Gynec. *98* 1057 1967
Moir C Edinburgh M.J *41* (Trans Edinb Obst. Soc. p 93) 1934
Posse N Acta obst. et gynec scandinav *37* Suppl. 2, 1958
Robertson E. H J Obst. & Gynec. Brit. Emp *46* 741 1939
Schultze G K. F Zentralbl. Gynäk *55* 3042, 1931

Received on May 20 1967

A. H Moawad s present address.

Dept of Obst. & Gyn. and Pharmacology
University of Alberta
EDMONTON Canada

some further attempts were made to operate on varicosities in pregnancy as for instance by *Cavanagh* and *Weinberg* (1954) and *Weissmann* and *Jenkins* (1956) In neither of these papers were obstetrical complications reported

Greenstone Heringman and *Masell* (1957) compared the results of identical operations made on pregnant and non pregnant women. They found unsatisfactory results after phlebectomy in only 4.5 per cent of the non pregnant women, whereas the final outcome of the operation in pregnant women was 72 per cent unsatisfactory They concluded that operations for varicosis in pregnancy should be done only on very pressing indications. Even then, only a limited emergency operation as a first stage procedure was advocated.

Thus the situation appears to be that all authors agree that an operation of the extent of high ligation and stripping is well tolerated by the pregnant woman. On the other hand, the phlebological results are somewhat debatable.

This is however not astonishing Recent experience with varicose veins demonstrates clearly that high ligature and stripping as sole measures usually are insufficient. Very often separate incisions for removal of varicose clusters must be done also and in cases of perforator incompetence these veins must be dealt with It is notable that in the reported series such procedures are never mentioned. Moreover it is likely that at least some of the authors quoted used the now old fashioned method of simple ligature of the long saphenous vein through a small incision in the groin. Modern treatment of varicose veins includes thorough dissection of the oval fossa with severing of all branches to the saphenous vein and the femoral vein within this area. It might very well be true that surgeons have been less inclined towards radical procedures during pregnancy mainly from fear of causing damage to the foetus (*Pflug*, 1967)

Apparently most of the patients in the series referred to were not operated upon using radical procedures possibly with the exception of the cases reported by *Dodd* who already at that time was a champion of radical venous surgery Since the modern operation for varicose veins unquestionable is a much larger procedure than the old intervention a double problem arises (1) Do

pregnant women tolerate the new radical surgery for venous insufficiency of the leg? and (2) What are the results after such measures?

Some women suffering from varicose veins in pregnancy have only minor disturbance. Others do not develop troublesome symptoms until the third trimester These factors give rise to further questions (3) Should all patients be operated upon? and (4) What measures can be taken if operation, for one reason or other is considered not necessary or postponed until after deli very? It is the aim of this paper to discuss these four questions

Material and methods

The problem of venous insufficiency of the leg was observed in 160 legs of 107 pregnant women. The average age was 31 years, with a range of 20 to 44 years.

Indications for operation during pregnancy 89 legs were treated conservatively The main reason for this choice was that the varicose state did not bother the patient very much. Among these legs were 34 which might be characterized as sites for angiectids (in German "Besenreiser-varizen") These angiological disturbances cannot be considered as true venous insufficiency They were described by *Fried Perlstein* and *Wagner* (1956) as a small superficial, intradermal raised, sharply circumscribed clump of bluish vessels and were taken as an expression of the disturbed hormonal balance during pregnancy In the remaining 55 legs there was a state of true venous incompetence. In 40 legs there was moderate inconvenience. In 15 legs there was more pronounced trouble but in these cases operation was postponed until after delivery since the patient did not attend until the latter months of pregnancy

It follows from the above that almost all women with varices which did bother them—by heaviness pain in the legs oedema or eczema of the hypostatic type—and who requested treatment during the first two trimesters were operated upon. 71 legs were treated surgically The time of operation in relation to the month of gestation is given in Table 1. In ten legs previous unsuccessful operations had been performed between 4 and 14 years earlier

Table I *Time of Operation and Period of Gestation*

Pregnancy-weeks	1–4	5–8	9–12	13–16	17 20	21–24
Number of operations	2	8	18	37	5	1

Table II. *Venous Insufficiency in 71 Operated Legs*

I Legs previously operated on		10
Recurrent insuff of long saphenous vein	6	
Idem+perforator insufficiency	3	
Idem+insuff of short saphenous vein	1	
II Legs not previously operated on		61
Extensive insuff of long saph. vein only	10	
Moderate insuff of do	6	
Insuff of long and short saph veins	5	
Extensive insuff of long saph vein+perf insuff	21	
Moderate insuff of do do	13	
Insuff of short saphenous vein only	3	
Insuff of perforators only	3	

The distribution and degree of the venous insufficiency in these legs and in the primarily operated legs are shown in Table II.

Operation All patients were operated upon under general anesthesia after pre-medication with atropine. Four patients were kept in hospital the others were treated as outpatients There was no special reason for hospital admission in the 4 cases except that in the beginning of our series we admitted all patients with varicose veins a practice that was later abandoned (*Hæger* 1967 b) *All operations during pregnancy were as radical as if the patient had not been pregnant* according to the general rules of the clinic as described by *Hæger* (1966 a b) The postopera tive course was checked at two or three visits to the clinic usually with one week s interval. The nature and extention of the surgical procedures are given in Table III

Methods of assessment At re-examination of the patient one or two years after the operation the obstetric course was checked with the patient. If it had been completely normal, according to the patient this was taken as correct and no further checking

Table III. *Types of Operation Performed on 71 Legs During Pregnancy*

Op perf in week	1–12	13–24	Total
High ligature + stripping + extensive removal of clusters	6	8	14
High ligature + stripping only or idem + removal of minor clusters	4	4	8
High ligature + stripping + perforat ligatur + remov of extensive clusters	7	16	23
High ligatur + stripping + perforat ligature, or idem + removal of minor clusters	7	7	14
High ligature + stripping + stripping of short saphenous vein	2	4	6
Stripping of short saphenous vein only	1	2	3
Perforator ligature subfascially	1	1	2
Perforator ligature extrafascially	0	1	1
Total	28	43	71

was done In questionable cases the clinic responsible for antenatal care and delivery was consulted, and the possible complications evaluated. In a special investigation 71 legs of non-pregnant women were selected as controls. Care was taken that the extension of the venous incompetence in this series was as similar as possible with that of the pregnant women treated by surgery The average age of the patients in this series was 33 years, with a range of 24–42 years As is seen in Table IV the operations performed on these non-pregnant women tally well with those performed during pregnancy (Table III)

All legs were inspected at least one year after surgery in both series and the patients subjective opinion of the outcome of the operation as well as the results of the objective examination were noted. The standards of assessment, characterized as excellent, good, fair and poor respectively were those used by *Haeger* (1966 a)

In the assessment of the results of the conservative treatment in cases of true venous incompetence the patient s subjective im

Table I *Time of Operation and Period of Gestation*

Pregnancy-weeks	1–4	5–8	9–12	13–16	17 20	21–24
Number of operations	2	8	18	37	5	1

Table II. *Venous Insufficiency in 71 Operated Legs*

I. Legs previously operated on		10
Recurrent insuff of long saphenous vein	6	
Idem+perforator insufficiency	3	
Idem+insuff of short saphenous vein	1	
II. Legs not previously operated on		61
Extensive insuff of long saph. vein only	10	
Moderate insuff of do	6	
Insuff of long and short saph. veins	5	
Extensive insuff of long saph vein+perf insuff	21	
Moderate insuff of do do	13	
Insuff of short saphenous vein only	3	
Insuff of perforators only	3	

The distribution and degree of the venous insufficiency in these legs and in the primarily operated legs are shown in Table II.

Operation All patients were operated upon under general anesthesia after pre-medication with atropine Four patients were kept in hospital the others were treated as outpatients. There was no special reason for hospital admission in the 4 cases except that in the beginning of our series we admitted all patients with varicose veins a practice that was later abandoned (*Hæger* 1967 b) *All operations during pregnancy were as radical as if the patient had not been pregnant* according to the general rules of the clinic as described by *Hæger* (1966 a, b) The postoperative course was checked at two or three visits to the clinic usually with one week s interval The nature and extention of the surgical procedures are given in Table III

Methods of assessment At re-examination of the patient one or two years after the operation the obstetric course was checked with the patient. If it had been completely normal according to the patient, this was taken as correct and no further checking

Table V *Results of Operations for Venous Insufficiency During Pregnancy*

	Result				Per cent
	Excellent	Good	Fair	Poor	satisfact.
Pregnant women operated on during first trimester	21	5	2	0	93 %
Pregnant women operated on during second trimester	33	5	3	2	90 %
Non-pregnant women	57	7	4	3	90 %

iron-deficiency anaemia of moderate degree developed. It was corrected by oral treatment with iron within one month of the operation. In the leg of this patient there was a slight delay of wound healing in two minor incisions, otherwise the postoperative state was normal.

The results of the operation as regards the venous situation are shown in Table V From the table it is evident that the results obtained after an observation time of one year in 20 cases, two years in 32 cases, three years in 10 cases four years in 7 cases and five years in 2 cases must be considered as satisfactory In the series of non-pregnant women observation time was at least three years in all cases.

In four women we had the opportunity to observe the state of the operated leg during a following pregnancy In two of these patients the results were excellent with no signs or symptoms of enous incompetence whatsoever In two cases some slight aricose eins appeared during the second trimester but did not cause the patient any inconvenience In both cases they disappeared after deliv ery without further treatment.

Results of conservative treatment

Patients with only disturbances of the angiectid type without other symptoms were given no treatment at all except the reassurance that the state would disappear within a few weeks after deli ery which also proved to be true in all cases.

The main symptoms in the cases of true enous insufficiency

Table IV *State of Veins in the Legs and Operations Performed in 71 Non-Pregnant Women*

Phlebological State	Operation Performed	No of Cases
Extensive insufficiency of long saphenous vein	High ligature + stripping + extensive removal of varicose clusters	19 (2)
Idem + perforator insufficiency	Idem + perforator ligature	19 (3)
Moderate insufficiency of long saphenous vein	High ligature + stripping only or idem + removal of minor varicose clusters	10 (1)
Idem + perforator insufficiency	Idem + perforator ligature	12 (2)
Insufficiency of short saphenous vein	Stripping of short saphenous vein	1
Perforator incompetence	Perforator ligature extrafascially	6 (2)
Idem	Perforator ligature subfascially	4
	Total	71 (10)

Figures in brackets indicate number of cases previously operated upon for varicose veins in the same leg

pression was considered to be of prime importance Taking into account the unavoidable vagueness of the system a broad grada tion in three categories was made. In addition, ankle and calf measurements were done each time the patient re visited the clinic.

Results of operative treatment

In the whole series of patients treated by surgery there were *no obstetrical complications* which in any way could be attributed to the anesthesia or the surgical intervention.

Postoperative complications were recorded in two patients. In one there was a minor infection in the groin incision. In the other bleeding during the operation was rather profuse and an

Table V *Results of Operations for Venous Insufficiency During Pregnancy*

	Result				Per cent
	Excellent	Good	Fair	Poor	satisfact
Pregnant women, operated on during first trimester	21	5	2	0	93 %
Pregnant women operated on during second trimester	33	5	3	2	90 %
Non-pregnant women	57	7	4	3	90 %

iron-deficiency anaemia of moderate degree developed. It was corrected by oral treatment with iron within one month of the operation. In the leg of this patient there was a slight delay of wound healing in two minor incisions otherwise the postopera tive state was normal.

The results of the operation as regards the venous situation are shown in Table V From the table it is evident that the results obtained after an observation time of one year in 20 cases, two years in 32 cases three years in 10 cases, four years in 7 cases and five years in 2 cases must be considered as satis factory In the series of non-pregnant women observation time was at least three years in all cases

In four women we had the opportunity to observe the state of the operated leg during a following pregnancy In two of these patients the results were excellent, with no signs or symptoms of venous incompetence whatsoever In two cases some slight aricose eins appeared during the second trimester but did not cause the patient any inconvenience In both cases they disappeared after delivery without further treatment.

Results of conservative treatment

Patients with only disturbances of the angiectid type without other symptoms were gi en no treatment at all except the reas surance that the state would disappear within a few weeks after delivery which also proved to be true in all cases.

The main symptoms in the cases of true venous insufficiency

Table IV *State of Veins in the Legs and Operations Performed in 71 Non-Pregnant Women*

Phlebological State	Operation Performed	No of Cases
Extensive insufficiency of long saphenous vein	High ligature + stripping + extensive removal of varicose clusters	19 (2)
Idem + perforator insufficiency	Idem + perforator ligature	19 (3)
Moderate insufficiency of long saphenous vein	High ligature + stripping only or idem + removal of minor varicose clusters	10 (1)
Idem + perforator insufficiency	Idem + perforator ligature	12 (2)
Insufficiency of short saphenous vein	Stripping of short saphenous vein	1
Perforator incompetence	Perforator ligature extrafascially	6 (2)
Idem	Perforator ligature subfascially	4
	Total	71 (10)

Figures in brackets indicate number of cases previously operated upon for varicose veins in the same leg

pression was considered to be of prime importance Taking into account the unavoidable vagueness of the system a broad grada tion in three categories was made In addition, ankle and calf measurements were done each time the patient re-visited the clinic.

Results of operative treatment

In the whole series of patients treated by surgery there were *no obstetrical complications* which in any way could be attributed to the anesthesia or the surgical intervention

Postoperative complications were recorded in two patients. In one there was a minor infection in the groin incision. In the other bleeding during the operation was rather profuse and an

were foot, ankle and leg oedema, pain in the legs and a feeling of heaviness All patients were given hydroflumethiazine (Vergonil® Ferrosan) in a dose of 25 mg daily This substance acts as a mild diuretic. In mild cases supportive stockings (Vogue Lycra® Malmö Strumpfabrik) were recommended, in more advanced cases the leg was bandaged with Lohmann s Dauer binde® and in severe cases either a Sigvaris® bandaging stocking or a Dalzoband®—Porelast® bandage was resorted to. The distribution of the use of each of these measures and the results are given in Table VI

From the table it is evident that conservative treatment as a rule caused relief from the symptoms and to some degree from the oedema of the legs as measured externally Both these parameters are included in the assessment of good, fair and poor respectively in Table VI. In no case was the end result considered as poor i.e all patients felt relief after one or two trials of bandaging.

Discussion

(1) *Do pregnant women tolerate radical surgery for venous incompetence?*

The answer to this question can be given as decidedly positive. Since in this rather large series of pregnant women there was no single obstetrical complication it appears that even extensive surgery for venous insufficiency can be done safely This is in agreement with earlier reports of surgery for varicose veins in pregnancy accepting that the extention of the surgical procedures in earlier series probably was more moderate.

In the literature concerning operations in pregnancy for other than obstetric reasons it is stated that the time of choice would be the second trimester since in the first trimester the danger of miscarriage would be greater (possibly owing to instability of hormone production) while in the third trimester the size of the uterus would make operation more difficult. This last reason however points to the assumption that the authors mainly had

Table VI *Results of Different Methods of Conservative Treatment of Legs with Venous Insufficiency in Pregnancy*

Degree of Symptoms at First Visit	No of Cases	Treatment at First Visit	Results			Further Treatment	Results		
			Good	Fair	Poor		Good	Fair	Poor
Very moderate	6	None	4	–	2	2 Lohmann	2	–	–
Moderate	34	20 support stockings	15	1	4	4 Lohmann	2	2	–
		14 Lohmann	7	2	5	5 Dalzoband	3	2	–
Severe	15	7 Lohmann	3	2	2	2 Dalzoband	–	2	–
		4 Dalzoband	3	1	–	None	–	–	–
		4 Sigvaris	3	1	–	None	–	–	–

were foot, ankle and leg oedema, pain in the legs and a feeling of heaviness All patients were given hydroflumethazine (Vergonil® Ferrosan) in a dose of 25 mg daily This substance acts as a mild diuretic. In mild cases supportive stockings (Vogue Lycra® Malmö Strumpfabrik) were recommended, in more advanced cases the leg was bandaged with Lohmann's Dauerbinde® and in severe cases either a Sigvaris® bandaging stocking or a Dalzoband®—Porelast® bandage was resorted to The distribution of the use of each of these measures and the results are given in Table VI.

From the table it is evident that conservative treatment as a rule caused relief from the symptoms and to some degree from the oedema of the legs, as measured externally Both these parameters are included in the assessment of good, fair and poor respectively in Table VI In no case was the end result considered as poor i.e all patients felt relief after one or two trials of bandaging.

Discussion

(1) *Do pregnant women tolerate radical surgery for venous incompetence?*

The answer to this question can be given as decidedly positive Since in this rather large series of pregnant women there was no single obstetrical complication it appears that even extensive surgery for venous insufficiency can be done safely This is in agreement with earlier reports of surgery for varicose veins in pregnancy accepting that the extension of the surgical procedures in earlier series probably was more moderate.

In the literature concerning operations in pregnancy for other than obstetric reasons it is stated that the time of choice would be the second trimester since in the first trimester the danger of miscarriage would be greater (possibly owing to instability of hormone production) while in the third trimester the size of the uterus would make operation more difficult. This last reason however points to the assumption that the authors mainly had

laparotomies in mind As is seen in Table III 28 out of 71 of our operations were performed during the first 12 weeks of preg nancy, in some instances even before the patient had realized she was pregnant. Judging from our series we have no objection to operating for venous insufficiency in the first trimester

(2) *What are the results of surgery for venous insufficiency performed during pregnancy?*

Our results must be considered satisfactory (Table V) As is demonstrated in that table there was no difference between the long term results of operations performed during pregnancy and similar operations in an almost identical series of non pregnant women. Neither were there any differences in the final outcome of the operations performed during the first and second tri mester respectively The results observed in this series are similar to those reported from a larger series from this clinic (*Hæger* 1966 a) They also agree with results from other clinics specializ ing in phlebological surgery (for references see *Hæger* 1966 a)

In some of the series reported earlier the long term phlebological results were bad. Our results contradict the assumption that this might be due to unfavourable conditions caused by the pregnant state *per se* It is much more likely that those bad results were due to the hesitation of surgeons to perform radical surgery during pregnancy There is a striking parallel between those early results obtained from operations during pregnancy and ours on one side and earlier non radical operations and present radical technique on the other

In an earlier report (*Hæger* 1966 a) it was stressed that the final results of operations for varicose veins were far better if the radical surgeon is allowed to start on a leg which has not been the subject of earlier non radical surgery Also for this reason the proposal by *Greenstone et al.* (1957) that in pressing situations only a limited procedure should be done as a first stage operation must be rejected. We think it important in all patients including women in pregnancy that if an operation for varicosities or per forator incompetence is decided upon it should be done in the best possible and most radical manner

(3) *Should all pregnant women with venous insufficiency be operated upon?*

It is true that many of the varicosities seen during pregnancy disappear after delivery. Experience from thousands of patients shows, however, that in the majority of cases the varicose state re-appears sooner or later (*Hæger* to be published). If a patient suffers from varicosities, particularly if this state was present prior to pregnancy and, in addition, the patient is disturbed by her venous insufficiency, we think that surgical operation is not only permissible but indicated. We do not, however, press our indications *in absurdum*. Thus patients with only slight symptoms may very well be treated by conservative means. Further we hesitate to operate upon patients even with severe symptoms in the third trimester of pregnancy. The reason for this is not so much fear from obstetrical complications but rather the knowledge that the aggravation resulting from pregnancy is near it's end, and that conservati e treatment in most cases gives temporary relief. Another point of interest in the discussion whether or not an operation is advisable is the opinion of the woman concerned. It should be noted that all women treated in our clinic were given the choice of operation or not (except those in late pregnancy). The high rate of operations suggests that the opinion of the women tends towards radical relief, *i.e.* operation.

It shall not be denied that there is an alternative to operation for the relief of troublesome varicose veins, *i.e.* sclerotherapy. Such treatment is recommended by many leading phlebologists, as for instance *Biegeleisen* (1936), *Leu* (1961), *Fegan* (1964) and *Sigg* (1967). Our experience, however, is that injected varicose veins have a disturbingly high rate of recurrence. This is true also for those injected during pregnancy. Our reasons for preferring operation were recently stated (*Hæger* 1967 a, *Arnoldi et al.* 1967 a, b) and shall not be repeated here. Let it only be stressed that in our experience the final outcome of radical operation for varices was just as bad after previous sclerotherapy as after previous inadequate operation (*Hæger* 1966 a). It follows that we refuse sclerotherapy even as a temporary measure during pregnancy.

laparotomies in mind. As is seen in Table III 28 out of 71 of our operations were performed during the first 12 weeks of pregnancy m some instances even before the patient had realized she was pregnant. Judging from our series we have no objection to operating for venous insufficiency in the first trimester

(2) *What are the results of surgery for venous insufficiency performed during pregnancy?*

Our results must be considered satisfactory (Table V) As is demonstrated in that table there was no difference between the long term results of operations performed during pregnancy and similar operations in an almost identical series of non-pregnant women. Neither were there any differences in the final outcome of the operations performed during the first and second tri mester respectively The results observed in this series are similar to those reported from a larger series from this clinic (*Hæger* 1966 a) They also agree with results from other clinics specializ ing in phlebological surgery (for references see *Hæger* 1966 a)

In some of the series reported earlier the long term phlebological results were bad. Our results contradict the assumption that this might be due to unfavourable conditions caused by the pregnant state *per se* It is much more likely that those bad results were due to the hesitation of surgeons to perform radical surgery during pregnancy There is a striking parallel between those early results obtained from operations during pregnancy and ours on one side and earlier non radical operations and present radical technique on the other

In an earlier report (*Hæger* 1966 a) it was stressed that the final results of operations for varicose veins were far better if the radical surgeon is allowed to start on a leg which has not been the subject of earlier non radical surgery Also for this reason the proposal by *Greenstone et al* (1957) that in pressing situations only a limited procedure should be done as a first stage operation must be rejected. We think it important in all patients including women in pregnancy that if an operation for varicosities or perforator incompetence is decided upon it should be done in the best possible and most radical manner

(3) *Should all pregnant women with venous insufficiency be operated upon?*

It is true that many of the varicosities seen during pregnancy disappear after delivery Experience from thousands of patients shows however that in the majority of cases the varicose state re appears sooner or later (*Hager* to be published) If a patient suffers from varicosities particularly if this state was present prior to pregnancy and, in addition, the patient is disturbed by her venous insufficiency we think that surgical operation is not only permissible but indicated. We do not, however press our indications *in absurdum*. Thus patients with only slight symptoms may very well be treated by conservative means. Further we hesitate to operate upon patients even with severe symptoms in the third trimester of pregnancy The reason for this is not so much fear from obstetrical complications but rather the know ledge that the aggravation resulting from pregnancy is near it s end, and that conservative treatment in most cases gives temporary relief Another point of interest in the discussion whether or not an operation is advisable is the opinion of the woman concerned. It should be noted that all women treated in our clinic were given the choice of operation or not (except those in late pregnancy) The high rate of operations suggests that the opinion of the women tends towards radical relief *i.e* operation.

It shall not be denied that there is an alternative to operation for the relief of troublesome varicose veins *i.e* sclerotherapy Such treatment is recommended by many leading phlebologists as for instance *Biegeleisen* (1936) *Leu* (1961) *Fegan* (1964) and *Sigg* (1967) Our experience however is that injected varicose veins have a disturbingly high rate of recurrence. This is true also for those injected during pregnancy Our reasons for preferring operation were recently stated (*Hager* 1967 a, *Arnoldi et al.* 1967 a, b) and shall not be repeated here. Let it only be stressed that in our experience the final outcome of radical operation for varices was just as bad after previous sclerotherapy as after previous inadequate operation (*Hager* 1966 a) It follows that we refuse sclerotherapy even as a temporary measure during pregnancy

laparotomies in mind. As is seen in Table III 28 out of 71 of our operations were performed during the first 12 weeks of pregnancy in some instances even before the patient had realized she was pregnant. Judging from our series we have no objection to operating for venous insufficiency in the first trimester

(2) *What are the results of surgery for venous insufficiency performed during pregnancy?*

Our results must be considered satisfactory (Table V) As is demonstrated in that table there was no difference between the long term results of operations performed during pregnancy, and similar operations *in an almost identical series* of *non pregnant* women. Neither were there any differences in the final outcome of the operations performed during the first and second tri mester respectively The results observed in this series are similar to those reported from a larger series from this clinic (*Hæger* *1966 a*) They also agree with *results* from other clinics specializ ing in phlebological surgery (for references see *Hæger* 1966 a)

In some of the series reported earlier the long term phlebological results were bad Our results contradict the assumption that this might be due to unfavourable conditions caused by the pregnant state *per se* It is much more likely that those bad results were due to the hesitation of surgeons to perform radical surgery during pregnancy There is a striking parallel between those early results obtained from operations during pregnancy and ours on one side and earlier non-radical operations and present radical technique on the other

In an earlier report (*Hæger* 1966 a) it was stressed that the final results of operations for varicose veins were far better if the radical surgeon is allowed to start on a leg which has not been the subject of earlier non radical surgery Also for this reason the proposal by *Greenstone et al.* (1957) that in pressing situations only a limited procedure should be done as a first stage operation must be rejected. We think it important in all patients including women in pregnancy that if an operation for varicosities or per forator incompetence is decided upon it should be done in the best possible and most radical manner

SUMMARY

A series of 160 legs with venous insufficiency in 107 pregnant women is presented. In 34 legs with angiectids no treatment was given, and the angiological disturbance disappeared after delivery in all cases. In 55 legs conservative treatment, consisting of diuretics and various types of compression bandages or stockings was resorted to. In 71 legs radical operation, including high ligature phlebectomy by stripping, removal of varicose clusters and, if necessary ligature of the medial ankle perforators, was done. There were no obstetric complications and the long-term results were equal to those of a similar series of women operated upon in the non-pregnant state.

REFERENCES

Arnoldi C C *Bauer* G *Gjöres* J E *Göthman* B. and *Hager* K. Läkartidn 64 48 1967
Ibidem 64 481 1967 b
Biegeleisen H L Angiology 5 64 1954
Birger I Zbl Phlebologie 6 152, 1967
Bohek T Ibidem 6 149 1967
Brenger F *Sepp*, K. and *Storm* H Ther Umschau 16 7 1959
Cavanagh M J and *Weinberg*, P C U S Armed Forces M. J 5 1619 1954
Dodd, H Lancet I 606 1949
Fegan W G Am Heart J 68 757 1984
Fried, P H *Perlstein* P F and *Wagner* F B. AMA Arch Surg. 72 253 1956
Greenstone S M *Heringman* E. C and *Massell* T B Calif Med. 87 363 1957
Hager K J Cardiovasc Surg 2 367 1961
Angiology 15 417 1984
Acta Chir Scand 131 38 1966
Venous and lymphatic disorders of the leg Scandinavian University Books, Lund 1966 b
Konservativ behandling vid perifera kärlsjukdomar En översikt. AB Häusles skriftserie, Göteborg 1966
Vasc Dis In print
Acta Chir Scand T be published
Hamilton H G *Pittam* R F and *Higgins* R S South M. J 42 608 1949
Lew H J Zbl Phlebologie I 46 1951
McPheeters H O Surg Gyn Obst. 81 355, 1945
Jones Lancet 69 2, 1949

(4) *Are our conservative methods of value?*

From Table VI it appears that in the majority of cases conserva tive treatment in one or another form gives temporary relief to the pregnant woman. In 29 cases simple measures such as support stockings or bandaging with a good elastic bandage gave good results In 13 cases a more comprehensive but still rather simple bandaging method was resorted to with good or fair re sults From the table it is also evident that if one type of therapy does not yield the desired response there usually is hope of im provement using methods with better compression.

For the relief of oedema we resorted to a very small dose of diuretics In earlier studies we have demonstrated the beneficial effect of different diuretics in cases of hypostatic oedema (*Hæger* 1961 1964) In those studies we used principally about the double equidiuretic dose of chlorthalidon (Hygroton® Geigy) or polythiazide (Renese® Pfizer) Using those drugs in higher doses we found hypopotassaemia in about 5 per cent of the cases independently of whether potassium was given simultaneously or not With the dose of hydrofluomethazine used in these series there was much lower incidence of hypopotassaemia (*Hæger* 1966 c) and this was our main reason for the choice of this drug in a low dose during pregnancy In cases of gross oedema however we believe that any other diuretic may be used, provided that a check is made from time to time of the level of serum potassium. In none of our cases treated with diuretics was there any complications attributable to the drug As far as we know there has never been reported any damage to the foetus after the use of thiazide drugs in pregnancy

In conclusion we propose that radical operation for varicose veins and perforator incompetence is a safe and desirable procedure during the first two trimesters of pregnancy In cases with only slight inconvenience or in patients presenting themselves in late pregnancy several conservative measures may give temporary relief

SUMMARY

A series of 160 legs with venous insufficiency in 107 pregnant women is presented. In 34 legs with angiectids no treatment was given, and the angiological disturbance disappeared after delivery in all cases. In 55 legs conservative treatment, consisting of diuretics and various types of compression bandages or stockings was resorted to. In 71 legs radical operation, including high ligature, phlebectomy by stripping, removal of varicose clusters, and, if necessary ligature of the medial ankle perforators was done. There were no obstetric complications and the long term results were equal to those of a similar series of women operated upon in the non-pregnant state.

REFERENCES

Arnoldi C C *Bauer* G *Gjöres* J E *Göthman* B and *Hagger* K. Läkartidn. 64 48 1967

Ibidem 64 481 1967 b

Burgielsson H L Angiology 5 84 1954

Birger I Zbl Phlebologie 6 152, 1967

Bobek T Ibidem 6 149 1967

Brenger F *Sogg*, K and *Stamm* H Ther Umschau 16 7 1959

Cavanagh M J and *Weinberg*, P C U S Armed Forces M J 5 1619 1954

Dodd, H Lancet I 606, 1949

Fegan W G Am Heart J 68 757 1954

Fried P H *Perlstein* P K. and *Wagner* F B AMA Arch Surg 72 253 1956

Greenstone S M *Heringman* E C and *Massell* T B Calif Med 87 363 1957

Hagger K J Cardiovasc Surg 2 367 1961

Angiology 15 417 1964

Acta Chir Scand 131 38 1966

Venous and lymphatic disorders of the leg. Scandinavian University Books Lund 1966 b

Konservativ behandling vid perifera kärlsjukdomar En översikt. AB Hässles skriftserie Göteborg 1966

Vasc Dis In print

Acta Chir Scand T be published

Hamilton H G *Pittman* R F and *Higgins* R S South M J 42 608 1949

Lee H J Zbl Phlebologie 1 48 1971

McPheeters H O Surg Gyn Obst 81 355 1945

Journ Lancet 69 2, 1949

(4) *Are our conservative methods of value?*

From Table VI it appears that in the majority of cases conservative treatment in one or another form gives temporary relief to the pregnant woman. In 29 cases simple measures such as support stockings or bandaging with a good elastic bandage gave good results In 13 cases a more comprehensive but still rather simple bandaging method was resorted to with good or fair results From the table it is also evident that if one type of therapy does not yield the desired response there usually is hope of improvement using methods with better compression.

For the relief of oedema we resorted to a very small dose of diuretics In earlier studies we have demonstrated the beneficial effect of different diuretics in cases of hypostatic oedema (*Hæger* 1961 1964) In those studies we used principally about the double equidiuretic dose of chlorthalidon (Hygroton® Geigy) or polythiazide (Renese® Pfizer) Using those drugs in higher doses we found hypopotassaemia in about 5 per cent of the cases independently of whether potassium was given simultaneously or not. With the dose of hydrofluomethazine used in these series there was much lower incidence of hypopotassaemia (*Hæger* 1966 c) and this was our main reason for the choice of this drug in a low dose during pregnancy In cases of gross oedema, however we believe that any other diuretic may be used provided that a check is made from time to time of the level of serum potassium In none of our cases treated with diuretics was there any complications attributable to the drug As far as we know there has never been reported any damage to the foetus after the use of thiazide drugs in pregnancy

In conclusion we propose that radical operation for varicose veins and perforator incompetence is a safe and desirable procedure during the first two trimesters of pregnancy In cases with only slight inconvenience or in patients presenting themselves in late pregnancy several conservative measures may give temporary relief

SUMMARY

A series of 160 legs with venous insufficiency in 107 pregnant women is presented. In 34 legs with angiectids no treatment was given, and the angiological disturbance disappeared after delivery in all cases. In 55 legs conservative treatment, consisting of diuretics and various types of compression bandages or stockings was resorted to. In 71 legs radical operation including high ligature, phlebectomy by stripping, removal of varicose clusters, and, if necessary ligature of the medial ankle perforators was done. There were no obstetric complications and the long-term results were equal to those of a similar series of women operated upon in the non-pregnant state.

REFERENCES

Arnoldi C C Bauer G Gjöres J E Göthman B and *Haeger K.* Läkartidn. 64 48 1967

Ibidem 64 481 1967 b

Bergelessen H L Angiology 5 64 1954

Birger I Zbl Phlebologie 6 152, 1967

Bobek T Ibidem 6 149 1967

Brunner F Stgg, K and Staemn H Ther Umschau 16 7 1959

Cavanagh M. J and *Weinberg, P C* U.S Armed Forces M. J 5 1619 1954

Dodd H Lancet 1 606 1949

Fegan W G Am Heart J 68 757 1964

Fried P H Perlman P K. and *Wagner F B* AMA Arch. Surg. 72 253, 1956

Greenstone S M Heitzman E C and *Massell T B* Calif Med. 87 363, 1957

Haeger K J Cardiovasc Surg 2 367 1961

Angiology 15 417 1964

Acta Chir Scand 131 38 1966

Venous and lymphatic disorders of the leg. Scandinavian University Books Lund 1966 b

Konservativ behandling vid perifera kärlsjukdomar En översikt. AB Hässle [illegible] Göteborg 1966

Vasc Dis In print

Acta Chir Scand To be published

Hamilton H G Pittman R F and *Higgins R S* South. M. J 42 608 1949

Lee H J Zbl Phlebologie 1 46 1951

McPheeters H O Surg Gyn Obst 81 355 1945

Journ Lancet 69 2, 1949

Peyton F W and *Loop F A* Am. J Obst. Gyn. *58* 318 1949
Pflug Zbl. Phlebologie *6* 152, 1967
Quattelbaum F W and *Hodgson Jane F* Surg. Gyn. Obst. *95* 330 1952
Sigg, K. Zbl. Phlebologie *6* 155 1967
Weissman R. E. and *Jenkins E. W.* J.A.M.A. *161* 1459 1956

Received on May 27 1967

Strede Nielsen, J and Moestrup, J K Acta obst et gynec scandinav 47 237 1968

From the Clinical-Physiological Laboratory (J Fabricius M.D) and the Department D of Obstetrics and Gynaecology (J J Jacobsen M.D and K. Kristoffersen M.D) Odense County and City Hospital Odense Denmark

FOETAL ELECTROCARDIOGRAPHIC STUDIES OF CARDIAC ARRHYTHMIAS AND THE HEART RATE

BY

J. STRÆDE NIELSEN AND J. K. MOESTRUP

The first recording of an electrocardiogram from a foetus (*Cremer* 1906) was carried out with an external electrode on the abdomen and an internal electrode either in the vagina or the rectum. It has since been possible to record a foetal electrocardiogram (ECG) with two abdominal electrodes (*Sondergaard* 1942) *Shenker* (1966) has summarized the results of the use of this method for the last 5 years. It is possible to record the foetal ECG with increasing certainty from approximately the 15th week of pregnancy It is thus possible to study the heart rate of the foetus at varying ages and under varying conditions both rapidly and without trauma, and to diagnose cardiac abnormalities *in utero* in larger numbers than earlier

The Method

An EMT 12 amplifier was used in connection with a 4-channel recorder mingograph 81 both from Elema-Schönander The system has a balanced input with an impedance of 100 mega-ohms and a specific suppression of 50 c.p.s. The maximum sensitivity is 10 microvolts per cm. It is possible to choose between 4 frequency filters and 10 time constants on the amplifier

After testing different types of electrodes we have found that the suction electrodes produced from German silver and which are normally used for precordial electrocardiography are the most

suitable for routine use. The electrodes are connected to the recorder by shielded cables and normal electrode paste was used.

The studies were carried out with the patients in a supine position. In pregnancies after 20 weeks maturity the 2 abdominal electrodes were placed in the midline one 5 cm above the symphysis and the other over the uterine fundus We have tried different positions for the patients and different positions for the electrodes as suggested by *Larks* (1961) but have not been able to obtain improved results Prior to the 20th week of pregnancy it has often been found more suitable to place the electrodes across the lower abdomen. As a rule both the high and low frequencies were suppressed as much as possible thus giving a maximum sensitivity between 10 and 20 c.p.s The QRS complex was thus considerably deformed. The P and T waves from the foetus were not recorded. In a number of studies the ECG of the foetus was followed on an oscilloscope permitting the optimal time of recording to be chosen.

In order to be certain of distinguishing between the foetal and maternal complexes in the signal from the abdominal electrodes a lead from the mother s extremities was recorded on channel 1 of the recorder On the 3 other channels the foetal ECG was recorded using different amplifications The paper speed used was 50 mm/sec. In order to obtain a good result it is important that the patient is relaxed, that the electrode paste is carefully rubbed into the abdominal skin and that the earthing is stable.

Material

The study includes a survey of 94 foetal ECG s obtained from pregnant women admitted to the gynaecological-obstetric department which only receives patients with pathological or potentially pathological pregnancies The reason for the examination has most frequently been obstetric problems (*Moestrup* and *Strøde Nielsen* 1967) whilst the remaining foetal ECG s were recorded in order to obtain a certain amount of normal data and in order to evaluate the accuracy of the method. It has not been possible to record the foetal ECG prior to the 15th week of pregnancy

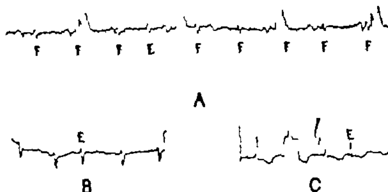


Fig 1 Premature beats in a foetus in the 38th week of pregnancy (A) and in the same child 1 day (B) and 1 week (C) after birth.

Results

Arrhythmias

On several occasions we have recorded the foetal ECG because of a suspicion of irregular heart action on auscultation but only in one case has it been possible to confirm the irregularity In addition to this we have found irregular foetal heart action accidentally on 4 occasions. Of these 5 arrhythmias we consider that the 3 could be termed premature beats and the other 2, sinus arrhythmias following sinus arrhythmia in the mother

Case 1 (J no 816/66–67)

This was 35 year old woman, pregnant for the 5th time who had a nontoxic goitre and wh had previously been subjected to Caesarean section because of transverse lie of the foetus A regular heart sound was heard during repeated control examinations from the 27th week of pregnancy In the 39th week of pregnancy the patient was admitted to the department because of exceptional foetal movements and irregular heart sounds with the loss of approximately every 4th beat A foetal ECG was taken (Fig. 1 A) The irregular heart action was not immediately recognized as being due to premature beats and Caesarean section was carried out for foetal distress The child weighed 3000 g and presented no signs of disease. The placenta was calcified, but otherwise normal An ECG of the child 1 day and 1 week after the birth showed few premature beats (fig 1 B and C) The child developed normally and auscultation at the age of 6 months showed normal heart action

suitable for routine use The electrodes are connected to the recorder by shielded cables and normal electrode paste was used.

The studies were carried out with the patients in a supine position. In pregnancies after 20 weeks maturity the 2 abdominal electrodes were placed in the midline, one 5 cm above the sym physis and the other over the uterine fundus. We have tried dif ferent positions for the patients and different positions for the electrodes as suggested by *Larks* (1961) but have not been able to obtain improved results Prior to the 20th week of pregnancy it has often been found more suitable to place the electrodes across the lower abdomen. As a rule both the high and low fre-quencies were suppressed as much as possible thus giving a maxi mum sensitivity between 10 and 20 c p s The QRS complex was thus considerably deformed The P and T waves from the foetus were not recorded. In a number of studies the ECG of the foetus was followed on an oscilloscope permitting the optimal time of recording to be chosen.

In order to be certain of distinguishing between the foetal and maternal complexes in the signal from the abdominal electrodes a lead from the mother s extremities was recorded on channel 1 of the recorder On the 3 other channels the foetal ECG was recorded using different amplifications The paper speed used was 50 mm/sec In order to obtain a good result it is important that the patient is relaxed, that the electrode paste is carefully rubbed into the abdominal skin and that the earthing is stable.

Material

The study includes a survey of 94 foetal ECG s obtained from pregnant women admitted to the gynaecological-obstetric depart ment which only receives patients with pathological or potentially pathological pregnancies The reason for the examination has most frequently been obstetric problems (*Moestrup* and *Stræde Nielsen* 1967) whilst the remaining foetal ECG s were recorded in order to obtain a certain amount of normal data and in order to evaluate the accuracy of the method It has not been possible to record the foetal ECG prior to the 15th week of pregnancy

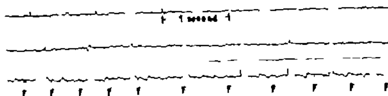


Fig. 4 Sinus arrhythmia in mother and foetus in the 24th week of pregnancy

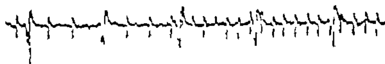


Fig. 5 Artefacts during attempts at obtaining foetal electrocardiogram.

Case no. 4 (*Journal no. 315/66–67*)

This was 24 year old woman in her 2nd pregnancy with frequent slight spinal haemorrhages from the 23rd week of pregnancy and until the birth. In the 4th week of pregnancy foetal ECG (Fig. 4) was taken as it was impossible to hear any heart sounds. This showed distinct, presumably respiratory sinus arrhythmia in the mother the rate varying from 70 to 102 beats per minute. The foetal heart action varied simultaneously from 91 to 142 beats per minute. One month later foetal ECG was taken owing to suspicion of breech presentation. The mother's heart action was still irregular but the child regular 7 weeks prior to the calculated time the birth took place following premature detachment of the placenta. The child was born in breech presentation, weighed 1400 g and was healthy.

A similar case however not quite so distinct, with presumed sinus arrhythmia in both the mother and child has been observed. The pregnancy ran normal course and ended in normal birth of healthy child. An attempt at reproducing this form of arrhythmia in other pregnant women by means of deep respiration has been unsuccessful.

Apart from the above mentioned arrhythmia we have recorded on 4 occasions irregular complexes of between 300 and 800 beats per minute similar to foetal ECG. An example of such so-called tachyarrhythmia is shown

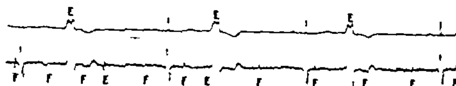


Fig. 2. Premature beats in a mother and foetus in the 18th week of pregnancy



Fig 3 Premature beats in a foetus in the 28th week of pregnancy

Case no 2 (Journal no 2440/65–66)

This was a 24 year old woman pregnant for the 4th time with 3 previous abortions Premature beats had been demonstrated in the mother on several occasions prior to the pregnancy without any sign of heart disease In order to demonstrate foetal life a foetal ECG was taken in the 18th week of pregnancy (Fig 2) This showed coupled premature beats in the mother and premature beats in the foetus followed by bradycardia. The mother was a non-smoker and she was receiving at that time the following drugs Chlorbutol aspirin, iron vitamins and phenobarbitone. Later in the pregnancy no irregular heart action was found in the foetus and the birth ran a normal course 3 months after occlusion of the internal os by means of Shirodkar suture because of cervical incompetence. The child was normal weighed 3300 g and showed no signs of heart disease Electrocardiogram of the mother and child 9 months after birth showed no abnormalities

Case no 3 (Journal no 1208/66–67)

This was a 29 year old primigravida with slight vaginal haemorrhages early in the pregnancy Owing to uncertain positioning of the foetus a foetal ECG was taken in the 28th week which showed premature beats without compensatory pauses (Fig 3) At one time in the 29th week of pregnancy irregular heart sounds were noticed on auscultation but otherwise the pregnancy ran a normal course The birth took place at the expected time but owing to a prolonged labour and meconium staining of the liquor a Caesarean section was carried out. The child weighed 4600 g and was healthy and developed normally

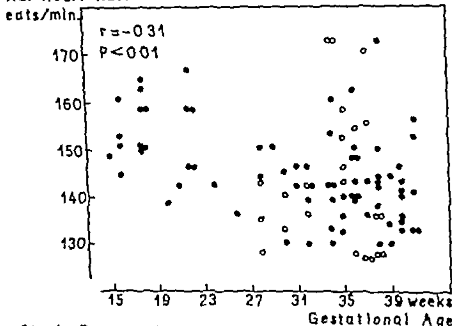


Fig. 7 The relation between the heart rate and the age of the foetus.

a heart rate of more than 150 per minute, whilst they only constitute one fourth of the group below this rate.

Discussion

As demonstrated by *Swartwoua et al.* (1961) and emphasized by *Shenker* (1966) it is possible to find on continuous recording of the heart rate in normal foetuses changes in rate occurring several times a minute amounting to approximately 10-15 beats per minute. Throughout later years in addition to this cases have been referred to with increasing frequency of arrhythmias which must be considered as abnormal.

Hon and Huang (1962) reviewed 25 cases of cardiac ar

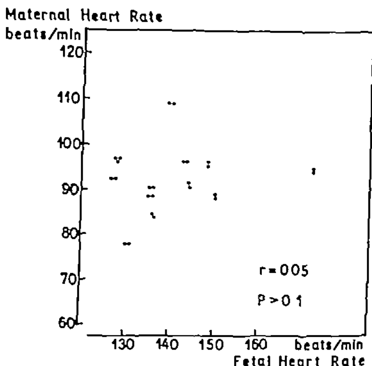


Fig 6 The relation between the heart rate of the mother and foetus

in Fig 5 In 3 of these cases it was possible either by moving the electrodes or by waiting a few minutes to obtain a normal foetal ECG The 4th patient in whom it was impossible to demonstrate any typical foetal ECG and also impossible to hear heart sounds gave birth 2 days later to a still-born, macerated child.

Frequency Measurements

It is possible to measure both the maternal and the foetal heart rate on a foetal ECG with considerable accuracy The average foetal heart rate in this series was 145 beats per minute (s 11) It can be seen from Fig 6 that there is no connection between the maternal and the foetal heart rate

The relation between the gestational age and the heart rate can be seen from Fig 7 A definite, though weak negative correlation can be seen suggesting a slight fall in the foetal heart rate throughout pregnancy The multiple pregnancies are marked by a circle on the figure It can be seen that after the 25th week of pregnancy these constitute more than half of the foetuses with

these so-called tachyarrhythmias are most probably artefacts, particularly if the rate is above 300 per min. (*Whitfield* 1966) We have considered all our rapid and irregular signals as artefacts, resulting from either an error in the apparatus, a poor electrode contact or muscle potentials from the mother

Hon (1963) established that the generally accepted average for the heart rate of the foetus is 140 beats per minute with a variation from 120–160 We found a slightly higher average, probably owing to the larger number of early pregnancies than in previous studies.

Many factors have been demonstrated such as cigarette smoking and fright (*Hellman et al.*, 1961) fever (*Sondergaard* 1942) and various drugs (*Solva* and *Salmi* 1959 *Hon* and *Huang*, 1962 *Lauro* and *Andriani* 1963) effecting both the heart rate of the mother and the foetus. We find, however similar to *Vara* and *Halminen* (1946) that under normal conditions and at rest the two heart rates are independent of each other

According to statements by *Bernstine* (1961) and *Shenker* (1966) the majority of studies do not show any signs of the foetal heart rate falling with increasing gestational age. *Lamkee et al* (1962) found, however a fall in the rate throughout pregnancy of the same magnitude as that in our material. The cause is probably that both series include early and late pregnancies Whether the heart rate of twins differs from that of others has not been mentioned previously

SUMMARY

Of 94 foetal ECG s which were recorded on women with pathological or potentially pathological pregnancies, 5 showed definite cardiac arrhythmias Three of these resulted from premature beats of no clinical importance The other two were considered to be examples of sinus arrhythmia, following a sinus arrhythmia in the mother The cause and importance of this is unknown. Artefacts can simulate tachyarrhythmia in the foetus.

The a erage heart rate in the foetus was found to be 145 per minut The heart rate of the mother and the foetus showed no mutual dependence. The relation between the gestational age

rhythmias in foetuses They were considered as sinus pauses, premature beats and escape beats. In 24 cases the birth ran a normal course, and the children were healthy whilst 1 premature child died during birth Arrhythmia was found in 2 of the children in the 2nd postnatal day One case of bigeminal rhythm in a foetus has been described by *Freistadt* (1962) The birth was normal, the child had a regular pulse and showed no signs of disease. *Sorland et al* (1963) described arrhythmia in a foetus which was observed by auscultation. A postnatal ECG showed supraventricular premature beats which disappeared when the child was 6 months old. *Millican et al* (1966) found, amongst 307 foetuses, 25 with abnormal rhythm of which only one had paroxysmal tachycardia of clinical importance Arrhythmia was also demonstrated postnatally in 2 of the children.

Our first 3 arrhythmias appear to have been premature beats. In the first case the Caesarean section could have been avoided if the nature of the arrhythmia had been recognized immediately A similar situation was mentioned by *Hon* and *Huang* (1967) The majority of arrhythmias have been seen in the last trimester particularly about the time of birth. Our second case was recorded in the 18th week of pregnancy and is of interest because the mother also had premature beats. It is tempting to assume a common origin but it has been impossible to demonstrate this. The foetal bradycardia following premature beats in both the mother and child is obvious but of unknown cause

Arrhythmia in the foetus following a respiratory sinus arrhythmia in the mother has not been previously reported. *Stander* and *Barden* (1964) found that the mother s deep respiration during sleep was accompanied by changes in the heart rate of the child, presumably as a result of variations in the intrauterine pressure Additional studies are necessary in order to demonstrate whether the mother s respiration under certain conditions affects the heart rate of the foetus

The term tachyarrhythmia in unborn children has been mentioned by both *Bernstine* (1961) and *Shenker* (1966) It can be seen from their papers that both atrial flutter atrial fibrillation and paroxysmal tachycardia can give rise to rapid and possibly irregular foetal ECG s It is stated however that the majority of

and heart rate showed a slight fall throughout the pregnancy A *heart rate above 150 per minute was found more frequently* in twins than in others

REFERENCES

Bernstine R. L. Fetal electrocardiography and electroencephalography
Cremer M Münch Med. Wschr 53 811 1906
Charles C. Thomas Springfield, Illinois 1961
Freistadt H Amer J Obstet. Gynec. 84 13 1962
Hellman L M Johnson H L Tolles W E and *Jones E H* Amer J Obstet. Gynec. 82 1055 1961
Hon E H and *Huang, H S* Obstet and Gynec. 20 81 1962
Hon E H Obstet. and Gynec. 22 137 1963
Larks S D Fetal electrocardiography Charles C. Thomas Springfield Illinois 1961
Lamkee M J Huntington H W and *de Alvarez R. R.* Amer J Obstet. Gynec. 83 1622 1962
Lauro V and *Andriani A.* Arch Obstet. Ginec. 68 672 1963
Millican E. Urbach J R. Carrington E. R. and *Lambert R. L.* Amer J Obstet. Gynec. 96 565 1966
Moestrup J K. and *Stræde Nielsen J* Nord. Med. 78 1086 1967
Shenker L. Obstet. gynec. Surv 21 367 1966
Soiva K. and *Salmi A.* Ann. Chir Gynaec Fenn 48 287 1959
Stander R. W and *Barden T P* Neb St. med. J 49 259 1964
Swartwout J R. Campbell W E. and *Williams L G* Amer J Obstet. Gynec. 82 301 1961
Sondergaard T Ugeskr Læg 104 775 1942
Sorland S Torp K. H and *Jenssen J T* norske Lægeforen 83 1654 1963
Vara P and *Halminen E* Acta obstet. gynec scand. 26 249 1946
Whitfield C R. Amer J Obstet. Gynec 95 669 1966

Received on June 12, 1967

Törnqvist Åke, Acta obst. et gynec. scandinav. 47: 257, 1968

From the Department of Obstetrics and Gynaecology (Professor S. Brody)
University of Göteborg, Sweden

THE EFFECTS OF EXOGENOUS HISTAMINE ON THE PULSE RATE IN PREGNANT AND NON-PREGNANT WOMEN BEFORE AND AFTER INHIBITION OF HISTAMINASE

BY

ÅKE TÖRNQVIST

The physiological effects of histamine during human pregnancy have been the subject of much study and discussion. Of considerable interest in this context is the increase in the plasma histaminase level (*Marcou et al.* 1938). *Ahlmark* (1944) found an increase in plasma histaminase activity during human pregnancy amounting to a thousand times that found in non-pregnant subjects. *Swanberg* (1950) showed that the decidual part of the human placenta was particularly rich in histaminase. He suggested that the increased plasma histaminase originated from that source.

The role of the plasma histaminase during human pregnancy is very intriguing. In spite of the high histaminase level pregnant women seem to react in very much the same way to exogenous histamine as non-pregnant subjects. This has been found for several histamine effects, e.g. metallic taste, dizziness and headache (*Janowitz* and *Grossman* 1949), eosinopenia (*Kullander* 1952), the size of the flare of the triple response (*Wicksell* 1949) and gastric secretion (*Clark* and *Tankel* 1954). *Lindberg* (1963) however, found a 50 per cent lower arterial blood concentration of ^{14}C histamine in pregnant women, as compared with non-pregnant women and males after intravenous histamine infusion. He suggested that the difference was caused by a more efficient histamine inactivation during pregnancy. His findings are, thus, somewhat at variance with the previous observations.

It is known that the circulatory effects of histamine particularly its influence on the heart rate are reproducible and related to the dose of histamine given In an investigation on the systemic effects of histamine in man *Weiss Robb* and *Ellis (1932)* found an increase in heart rate after both single injections and continuous intravenous infusions The response was rapid and the increased heart rate was fairly constant as long as the same infusion rate was maintained. It would seem that this effect of histamine would be more suitable to quantitative analysis of the histamine effect than other physiological functions used as criteria earlier

A valuable method of investigating an enzyme is to study it by means of a specific inhibitor The most specific inhibitor of histaminase is aminoguanidine (*Schuler* 1952) Its chemistry has been investigated by *Lieber* and *Smith* (1939) and comparative physiological actions of aminoguanidine and related substances have been studied by *Alles* (1926) Its toxicity is low Aminoguanidine given orally inhibits histaminase almost completely *(Lindell Roos Nilsson* and *Westling,* 1960) *Lindberg* and *Törnqvist* (1966) have demonstrated that aminoguanidine can inhibit histaminase *in vivo* during human pregnancy After inhibition the blood concentration of unchanged ^{14}C histamine during intravenous infusion to pregnant women rose to a non-pregnant level This is evidence that histaminase is active *in vivo* in pregnant women

The purpose of the present investigation has been to compare the effect of infused histamine on the pulse rate in pregnant and non pregnant women and in addition to study the responses after histaminase inhibition by means of aminoguanidine

Material

Thirty two physically healthy pregnant women were investigated. They were admitted to the clinic for legal abortion and were in the 15th to the 24th week of pregnancy counting from the first day of the last menstrual period. The mean length of pregnancy in these cases was 20 weeks. The ages ranged from 14 to 40 years with a mean of 26 years. The body weights ranged from 43 to 74

kg with a mean of 59 kg. Sixteen non-pregnant women with regular menstrual periods were investigated as well. Most of them belonged to the nursing staff. Their ages ranged from 21 to 47 years. The mean age was 32 years. The body weights of the non-pregnant women varied from 50 to 69 kg with a mean of 57 kg.

Methods

Infusion of histamine Histamine was infused through an indwelling needle in the long saphenous vein at the ankle. The infusion rates were held constant by means of a motor-driven syringe. By changing the motor speed different amounts of histamine per minute were infused. The histamine was dissolved in physiological saline (1 mg of histaminedihydro chloride E. Merck A G per 100 ml) After three minutes of infusion the pulse rate reached a fairly constant level. Histamine infusion periods of 6 minutes were chosen. Histamine-free intervals of 8 minutes were sufficient to restore basal heart rate. In the histamine-free intervals physiological saline solution was infused at a low speed to prevent clotting in the needle and the vein.

Estimation of the pulse rate The pulse rate was counted from a record of the volume pulsations in the right hand. A plethysmograph was used. The water temperature in the plethysmograph was held constant at 34 C. Care was taken to ascertain that the patients were kept in a comfortable position Light and temperature in the room were held constant as far as possible The arterial blood pressure was recorded in the left arm by the conventional sphygmomanometer cuff method and auscultation of Korotkow's sound. During the histamine infusions the blood pressure was recorded every 30 seconds and during the histamine-free intervals at least every 5 minutes. After the recording apparatus and the indwelling needle were fitted, the patients were kept in bed for 30 minutes before any recordings were made. The pulse rate during the histamine infusions was recorded continuously and was carried out on paper by means of a piston recorder from the 30th second to the end of the infusion. During the last three minutes of the histamine infusions pulse rate countings were done every

It is known that the circulatory effects of histamine particularly its influence on the heart rate are reproducible and related to the dose of histamine given In an investigation on the systemic effects of histamine in man *Weiss Robb* and *Ellis* (1932) found an increase in heart rate after both single injections and continuous intravenous infusions The response was rapid and the increased heart rate was fairly constant as long as the same infusion rate was maintained It would seem that this effect of histamine would be more suitable to quantitative analysis of the histamine effect than other physiological functions used as criteria earlier

A valuable method of investigating an enzyme is to study it by means of a specific inhibitor The most specific inhibitor of histaminase is aminoguanidine (*Schuler* 1952) Its chemistry has been investigated by *Lieber* and *Smith* (1939) and comparative physiological actions of aminoguanidine and related substances have been studied by *Alles* (1926) Its toxicity is low Aminoguanidine given orally inhibits histaminase almost completely (*Lindell Roos Nilsson* and *Westling*, 1960) *Lindberg* and *Törnqvist* (1966) have demonstrated that aminoguanidine can inhibit histaminase *in vivo* during human pregnancy After inhibition the blood concentration of unchanged ^{14}C histamine during intravenous infusion to pregnant women rose to a non-pregnant level This is evidence that histaminase is active *in vivo* in pregnant women

The purpose of the present investigation has been to compare the effect of infused histamine on the pulse rate in pregnant and non pregnant women and in addition to study the responses after histaminase inhibition by means of aminoguanidine

Material

Thirty two physically healthy pregnant women were investigated. They were admitted to the clinic for legal abortion and were in the 15th to the 24th week of pregnancy counting from the first day of the last menstrual period. The mean length of pregnancy in these cases was 20 weeks The ages ranged from 14 to 40 years with a mean of 26 years The body weights ranged from 43 to 74

Table I. *Histamine Infusion in Non-Pregnant Women*

Case	Increase in Pulse Rate before Histaminase Inhibition			Increase in Pulse Rate after Histaminase Inhibition		
	Infusion Rate of Histamine			Infusion Rate of Histamine		
	7	12	21	7	12	21
1	6 (73)	11 (75)	15 (74)	2 (67)	7 (74)	18 (72)
2	9 (83)	11 (81)	18 (81)	11 (83)	17 (82)	24 (81)
3	6 (73)	13 (71)	17 (72)	8 (68)	14 (74)	17 (73)
4	10 (61)	15 (58)	25 (59)	14 (58)	14 (63)	27 (61)
5	3 (73)	9 (78)	24 (66)	4 (64)	10 (72)	20 (67)
6	6 (78)	8 (76)	14 (76)	3 (70)	14 (70)	18 (70)
7	7 (64)	16 (60)	27 (55)	6 (53)	10 (58)	30 (52)
8	6 (72)	17 (66)	24 (65)	5 (70)	14 (70)	28 (67)
9	7 (71)	11 (66)	21 (61)	6 (68)	21 (61)	23 (68)
10	12 (59)	12 (59)	30 (60)	8 (53)	20 (56)	33 (54)
11	3 (63)	10 (60)	27 (61)	4 (65)	15 (61)	26 (65)
12	2 (76)	8 (74)	21 (70)	2 (71)	12 (71)	21 (70)
13	8 (81)	14 (79)	14 (81)	5 (76)	10 (73)	18 (76)
14	6 (60)	16 (54)	24 (56)	8 (58)	15 (57)	24 (59)
15	7 (81)	10 (83)	20 (81)	6 (81)	13 (82)	23 (82)
16	3 (83)	12 (74)	25 (77)	7 (78)	13 (74)	27 (73)
Mean	6 (72)	12 (70)	22 (69)	6 (68)	14 (69)	24 (68)

Infusion rate of histamine in μg per minute.
Pulse rate in beats per minute.
The figures in brackets indicate the pulse rate immediately before the histamine infusion.

The significances of differences between groups were tested by means of the Wilcoxon two-sample rank test (*Brownlee* 1961) As the same results were analysed in two respects with one testing method, the tests are not independent and a significance level of 2.5 per cent was chosen in each test, to ensure that the significance level of the combined tests was, at least, on the 5 per cent level

Results

Table I shows the increase in pulse rate of the nonpregnant control group at infusion rates of 7 12 and 21 micrograms of hista-

20th second from the continuous record. Every counting was done for 10 seconds The sum of 6 consecutive countings was cal culated In the same way the pulse rate during the histamine-free periods was counted from a two minute pulse rate record immediately before every histamine infusion The variation in the 10 second countings of the pulse rate did not exceed an average of two beats The variations were similar in the pregnant and the non pregnant women The difference between the pulse rate during histamine infusion and the pulse rate during the histamine-free periods was taken as a measure of the histamine response

Inhibition of histaminase Histaminase inhibition was achieved by means of injecting intramuscularly 0 2 mg of aminoguanidine sulphate (Eastman Organical Chemicals) per kg of body weight. The solution injected contained 10 mg of aminoguanidine sul phate per ml of sterilised water That a period of 30 minutes was found to be sufficient for complete inhibition of the histaminase will be discussed later (*Törnqvist* unpublished)

Infusion schedule To 10 pregnant women (cases 1–10) and to the non pregnant control group histamine was infused with infusion rates of 7 12 and 21 micrograms per minute before as well as after histaminase inhibition The sequence of the different infusions was at random Seven pregnant women (cases 11–17) received only 21 micrograms of histamine per minute and 5 women (cases 18–22) only 7 micrograms per minute before and after inhibition of histaminase To study if there was any lasting effect of histamine on the response of the pulse rate to histamine at a repeated histamine infusion 10 pregnant women were infused with 21 micrograms of histamine per minute on two occasions 30 minutes apart. In this group the histaminase inhibition was replaced by an intramuscular injection of physiological saline solution

Statistics

The responses of the pregnant and of the non-pregnant women, before inhibition of histaminase were compared The change in the effects of the histamine caused by inhibition of histaminase in the pregnant and the non pregnant groups was also compared.

Table I. Histamine Infusion in Non-Pregnant Women

Case	Increase in Pulse Rate before Histaminase Inhibition			Increase in Pulse Rate after Histaminase Inhibition		
	Infusion Rate of Histamine			Infusion Rate of Histamine		
	7	12	21	7	12	21
1	6 (73)	11 (75)	15 (74)	2 (67)	7 (74)	18 (72)
2	9 (83)	11 (81)	18 (81)	11 (83)	17 (82)	24 (81)
3	6 (73)	13 (71)	17 (72)	6 (68)	14 (74)	17 (73)
4	10 (61)	15 (58)	25 (59)	14 (58)	14 (63)	27 (61)
5	3 (73)	9 (78)	24 (66)	4 (64)	10 (72)	20 (67)
6	6 (78)	8 (76)	14 (76)	3 (70)	14 (70)	18 (70)
7	7 (64)	16 (60)	27 (55)	8 (53)	10 (58)	30 (52)
8	6 (72)	17 (66)	24 (66)	5 (70)	14 (70)	28 (67)
9	7 (71)	11 (66)	21 (61)	6 (68)	21 (61)	23 (68)
10	12 (59)	12 (59)	30 (60)	8 (53)	20 (56)	33 (54)
11	3 (63)	10 (60)	27 (61)	4 (65)	15 (61)	26 (65)
12	2 (76)	8 (74)	21 (70)	2 (71)	12 (71)	21 (70)
13	8 (81)	14 (79)	14 (81)	5 (76)	10 (73)	18 (76)
14	6 (60)	16 (54)	24 (56)	8 (58)	15 (57)	24 (59)
15	7 (81)	10 (83)	20 (81)	6 (81)	13 (82)	23 (82)
16	3 (83)	12 (74)	25 (77)	7 (78)	13 (74)	27 (73)
Mean	6 (72)	12 (70)	22 (69)	6 (68)	14 (69)	24 (68)

Infusion rate of histamine in μg per minute.
Pulse rate in beats per minute.
The figures in brackets indicate the pulse rate immediately before the histamine infusion.

The significances of differences between groups were tested by means of the Wilcoxon two-sample rank test (*Brownlee* 1961). As the same results were analysed in two respects with one testing method, the tests are not independent and a significance level of 2.5 per cent was chosen in each test, to ensure that the significance level of the combined tests was at least, on the 5 per cent level.

Results

Table I shows the increase in pulse rate of the nonpregnant control group at infusion rates of 7, 12 and 21 micrograms of hista

Table II. *Histamine Infusion in Pregnant Women*

Case	Increase in Pulse Rate before Histaminase Inhibition			Increase in Pulse Rate after Histaminase Inhibition		
	Infusion Rate of Histamine			Infusion Rate of Histamine		
	7	12	21	7	12	21
1	8 (96)	10 (94)	14 (96)	9 (96)	8 (90)	17 (90)
2	2 (90)	7 (89)	11 (89)	7 (88)	13 (90)	21 (89)
3	3 (108)	9 (109)	8 (106)	4 (102)	14 (102)	22 (101)
4	−1 (92)	14 (90)	15 (91)	8 (88)	14 (87)	25 (89)
5	7 (84)	8 (84)	17 (84)	10 (82)	25 (85)	30 (84)
6	4 (86)	10 (78)	16 (80)	9 (73)	13 (79)	18 (73)
7	7 (76)	10 (71)	20 (72)	10 (67)	23 (61)	29 (61)
8	3 (99)	10 (97)	13 (95)	4 (92)	15 (94)	19 (89)
9	0 (81)	11 (88)	10 (83)	5 (85)	9 (82)	20 (79)
10	6 (85)	9 (83)	13 (85)	1 (88)	9 (80)	20 (74)
11			14 (65)			28 (65)
12			14 (86)			21 (87)
13			10 (78)			16 (79)
14			22 (79)			27 (7[illegible])
15			20 (73)			27 (70)
16			19 (77)			31 (76)
17			10 (102)			16 (104)
18	1 (76)			13 (75)		
19	4 (84)			6 (86)		
20	−2 (92)			3 (96)		
21	1 (91)			5 (97)		
22	3 (76)			3 (78)		
Mean	3 (88)	10 (88)	15 (85)	6 (86)	14 (86)	23 (83)

Infusion rate of histamine in μg per minute.
Pulse rate in beats per minute.
The figures in brackets indicate the pulse rate at rest immediately before the histamine infusion.
indicates that no histamine infusion was done.

mine per minute. The increase in pulse rate was closely related to the dose of histamine. There was almost no additional average increase in pulse rate after inhibition of histaminase when identical doses of histamine were infused.

Table II shows the increase in pulse rate of the pregnant women under identical conditions. In this group there was also a close

Table III. Pregnant Women Infused with Histamine on Two Successive Occasions

Case	Increase in Pulse Rate During the First Histamine Infusion	Increase in Pulse Rate During the Second Histamine Infusion
1	19 (84)	21 (87)
2	15 (82)	7 (90)
3	19 (91)	14 (91)
4	26 (76)	23 (82)
5	17 (91)	19 (88)
6	17 (79)	14 (79)
7	12 (81)	20 (84)
8	12 (97)	6 (102)
9	12 (79)	14 (77)
10	7 (91)	10 (86)
Mean	16 (85)	15 (87)

Infusion rate of histamine 21 μg per minute.
Pulse rate in beats per minute.
Figures in brackets indicate the pulse rate at rest immediately before the histamine infusion

relation between the increase in pulse rate and the dose of histamine Before histaminase inhibition the response to histamine was not so pronounced as in the non-pregnant group. After inhibition of histaminase the response to histamine was increased almost to the non pregnant level.

Table III shows the increase in pulse rate of the pregnant women infused with two successive doses of 21 micrograms of histamine per minute without interposed inhibition of histaminase. In 5 subjects the increase in pulse rate was more pronounced during the second infusion of histamine In the other 5 cases, however the result was inversed According to a sign test (*Brownlee* 1961) no difference can be proved between the two infusions of histamine in this series.

The histamine responses before inhibition of histaminase in all pregnant and non-pregnant women were compared at the infusion rates of 7 and 21 micrograms of histamine per minute. The different increases in pulse rate in the pregnant and the non-pregnant cases were tested for statistical significance with the

Table IV *Statistical Analysis of the Increase in Pulse Rate Caused by Histamine Infusion in Pregnant and Non-Pregnant Women. The Wilcoxon Two-Sample Rank Test Was Used.*

	Infusion Rate of Histamine	
	7	21
Sum of rank for the pregnant women	176 (15)	446.5 (27)
Sum of rank for the non-pregnant women	320 (16)	499.5 (16)
Upper critical value for the one-tailed test at the 2.5 per cent level	306	431

The sum of rank for the non-pregnant women exceeds the critical value.
Infusion rate of histamine in micrograms per minute.
Figures in brackets indicate the number of cases at this infusion rate

Wilcoxon two-sample rank test (*Brownlee* 1961) The pregnant cases infused with 12 micrograms of histamine per minute were too few to allow statistical analysis with this test.

Table IV shows that the differences between the pregnant and the non-pregnant women are statistically significant and nothing contradicts the hypothesis that the non-pregnant women are more sensitive to infused histamine than the pregnant ones. The additional increases in the pulse rate caused by inhibition of histaminase during infusion of histamine, of the pregnant and the non

Table V *Statistical Analysis of the Additional Increase in Pulse Rate Caused by Histaminase Inhibition in Pregnant and Non-Pregnant Women During Histamine Infusion The Wilcoxon Two-Sample Rank Test Was Used.*

	Infusion Rate of Histamine	
	7	21
Sum of rank for the pregnant women	314 (15)	407 (17)
Sum of rank for the non-pregnant cases	1[illegible]2 (16)	154 (16)
Upper critical value for the one-tailed test at the 2.5 per cent level	290	344

The sum of rank for the non-pregnant women exceeds the critical value
Infusion rate of histamine in micrograms per minute.
Figures in brackets indicate the number of cases at this infusion rate

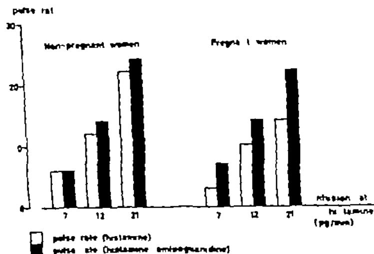


Fig 1 Graphical representation of the average increase in pulse rate during histamine infusion. The columns represent the non-pregnant subjects in table I and the pregnant women in tables II and III. The light columns indicate the increase in pulse rate before and the dark columns the increase after inhibition of histaminase. The increase in pulse rate is expressed as Δ pulse rate (beats per minute).

pregnant women were also compared. Histaminase inhibition gave in the pregnant subjects an additional average increase of three beats per minute at an infusion rate of 7 micrograms of histamine per minute and 8 beats per minute when 21 micrograms were infused. In the non-pregnant control group histaminase inhibition did not alter the histamine response appreciably To test the difference in pulse rate increase between the pregnant and the non-pregnant women for statistical significance the Wilcoxon two-sample rank test was applied. The results of the test are given in Table V The differences were statistically significant and there is reason to believe that histaminase inhibition increases the response of the pregnant women more than that of the non-pregnant ones Fig 1 is a graphical representation of the histamine response of both pregnant and non-pregnant women before and after inhibition of histaminase

The correlation between the pulse rate at rest and the increase

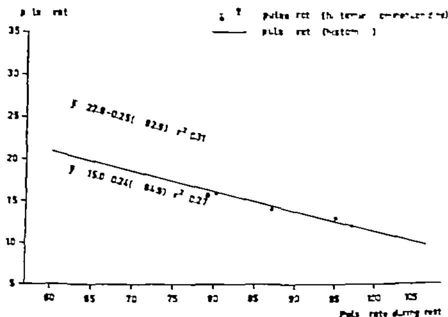


Fig. 2. The correlation between the pulse rate at rest and the increase in pulse rate (Δ pulse rate) during infusion of histamine in pregnant women. The infusion rate of histamine was 21 micrograms per minute. The figure includes all pregnant cases infused at this dose level.

in pulse rate during infusion of histamine was also investigated. The investigation was done for both pregnant and nonpregnant women at an infusion rate of 21 micrograms per minute In Fig 2 this correlation is given for the pregnant subjects before and after inhibition of histaminase A certain correlation exists. The lower the pulse rate at rest the higher the increase in pulse rate during histamine infusion Fig 3 shows a similar correlation for the non-pregnant women

No correlation was found between the increase in pulse rate during histamine infusion and the body weights of the patients. Nor did the arterial blood pressure in the left arm vary appreciably Small fluctuations of 5 to 10 mm Hg were observed.

Discussion

From the work of *Schayer* and co-workers (1955–1959) the principal catabolic pathways are now known. *Lindahl* and co-workers

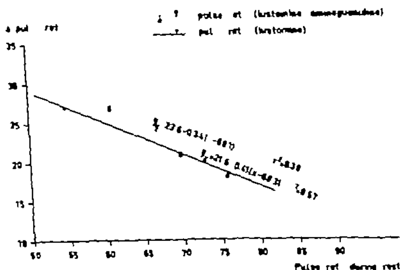


Fig. 3 The correlation between the pulse rate at rest and the increase in pulse rate (Δ pulse rate) during infusion of histamine in the non-pregnant women. The figure includes all non-pregnant cases infused at a dose rate of 21 micrograms of histamine per minute.

(1938) studied histamine inactivation with special reference to human pregnancy Schyer found in both human and animal investigations that histamine is metabolized by oxidative deamination of the side chain to imidazole-4(5)-acetic acid and by methylation of the ring nitrogen to 1-methyl-4-(β-aminoethyl) imidazole Histaminase investigated among others by *Best* (1929) is responsible for the oxidative deamination. The enzyme involved in the methylation has been called imidazole-N-methyl-transferase by *Brown Tomchick* and *Axelrod* (1959) and histamine-methyl-transferase by *Lindahl-Kiessling* (1960) Additional pathways for histamine catabolism appear to lack quantitative significance. Consequently it must be considered that aminoguanidine only inhibits the oxidative deamination of histamine and leaves the quantitatively important methylation intact. There is also reason to believe that the methylation in some cases of histaminase in-

hibition by means of aminoguanidine is compensatorily increased (*Lindberg* and *Törnqvist* 1966)

In this investigation the pulse rate was chosen as the parameter for the histamine response. The response to infused histamine was significantly higher in the non-pregnant than in the pregnant women This is at variance with earlier investigations indicating similar histamine sensitivity to exogenous histamine in pregnant and non pregnant women In none of these earlier investigations (*Janowitz and Grossman* 1949 *Kullander* 1952 *Wicksell* 1949 *Clark* and *Tankel* 1954) however was the pulse rate used as the parameter for the histamine response

In the non pregnant control group histaminase inhibition by means of aminoguanidine did not alter the histamine response appreciably In the pregnant cases however histaminase inhibition increased the histamine response The results at the infusion rate levels of 7 and 21 micrograms of histamine per minute were statistically significant. The individual increase in histamine sensitivity after histaminase inhibition varied considerably The intact, or possibly increased methylation of histamine might possibly explain this variation to some extent.

Lindberg (1963) proposed an explanation for the discrepancy between earlier reported investigations on the histamine response in pregnant and non-pregnant women and his own observation of a 50 per cent lower plasma histaminase level in pregnant, as compared with non pregnant women during histamine infusion. If the effector cells (e g vascular smooth muscle) during pregnancy had an increased sensitivity towards histamine the rise in plasma histaminase activity could possibly be regarded as a means for keeping the histamine response at a normal level. In the present investigation the histamine response increased after histaminase inhibition but it did not exceed that of the non-pregnant subjects. These results do not support Lindberg s hypothesis. If there had been a method to inhibit the histamine-methyl-transferase in man as well, there might have been an even more pronounced response to the infused histamine in the pregnant subjects.

From earlier investigations it is known that the heart rate at rest is increased during pregnancy The reports on the basal heart

rate during the second trimester of human pregnancy are very conflicting. Average basal heart rates ranging from 76 (*Widlund* 1945) to 98 beats per minute (*Bader Braunwald* and *Rose* 1955) have been reported. In the present investigation the average heart rate at rest was also considerably higher for the pregnant than for the non-pregnant cases There was also a correlation between the pulse rate at rest and the increase in pulse rate during infusion of histamine. This was true for both pregnant and non-pregnant cases. The lower the pulse rate at rest the higher the increase in pulse rate during histamine infusion. This fact, however did not explain the smaller histamine response in the pregnant women. After histaminase inhibition the pulse rate at rest was unaltered but the histamine response was considerably increased.

In a few pregnant subjects the pulse rate at rest, was rather high but there were no demonstrable signs of disturbed basal conditions. In some cases there was a slight decrease in pulse rate throughout the observation. According to *Alles* (1926) aminoguanidine produced a considerable decrease in heart rate in rabbits Owing to the investigative methods used *Alles* questioned his own results. In the present investigation there was no appreciable decrease in the pulse rate at rest after inhibition of histaminase by means of aminoguanidine

The fact that histaminase inhibition did not increase the pulse rate at rest in pregnant women is very interesting *Kahlson Rosengren* and *Westling* (1958) suggested that the increased histaminase activity during human pregnancy might possibly be a defense mechanism against endogenous (*e g* foetal) histamine production. A possible explanation of the results in the present investigation may be that in man there is no appreciable endogenous histamine production or if there is such a production it may be too small to give a measurable increase in the pulse rate. It is also possible that the histamine produced in the foetus, unlike exogenous histamine is largely methylated. *Lindberg, Lindell* and *Westling* (1963) found that histamine injected into the umbilical cord in connection with surgical induction of abortion, was largely methylated *Kahlson Rosengren* and *Westling* (1958) found an increased urinary histamine excretion in the pregnant rat. *Bjurd Lindberg* and *Westling* (1961 and 1964) found an in-

crease in urinary histamine during human pregnancy The in crease however was small compared with that in the pregnant rat. Furthermore aminoguanidine given orally did not increase the urinary histamine measurably in the pregnant woman. *Michell* (1956) on the contrary found an increase in urinary histamine excretion after injecting histamine subcutaneously to healthy men when aminoguanidine in sufficiently high doses was given orally It should be emphasized that the present study for obvious ethical reasons was undertaken on women in relatively early pregnancy It seems probable that the observed effects of the aminoguanidine might have been larger in the later stages of pregnancy when histaminase activity is higher It is also possible that the aminoguanidine then would have had an effect on the basal pulse rate since endogenous histamine production may be higher at a later stage of pregnancy (*Kahlson et al* 1958 *Bjurö et al* 1964 *Granerus Lindell* and *Westling*, unpublished)

The increase in histamine sensitivity after histaminase inhibi tion demonstrated in the pregnant women in the present investigation might seem too small to represent an almost complete inhibition of histaminase. If the results are compared with earlier studies on the histamine sensitivity of guinea pigs there is a cer tain resemblance *Lindell* and *Westling* (1954) studied the hista mine sensitivity of the bronchi and the urinary bladder in guinea pigs before and after histaminase inhibition and *Westling* (1956 and 1957) studied the potentiation of histamine effects on the respiration and the rectal temperature in guinea pigs under the same conditions In these investigations the potentiation of the histamine effects was mainly characterized by a prolongation of the response after inhibition of histaminase The maximal increase in sensitivity was about two-fold As the guinea pig is very sensitive to histamine the authors considered the potentiation of the histamine response to be small. Even if the potentiation of the histamine effect in the present investigation was small it was of statistical significance and suggests that the enzyme histaminase is active *in vivo* in the pregnant woman

SUMMARY

1 The response to histamine infused intravenously was studied in 32 pregnant and 16 non-pregnant women. The pulse rate was chosen as the parameter for the effect of the histamine.

2 To investigate if the enzyme histaminase modified the histamine effect in pregnant women, the response to infused histamine was also studied after specific histaminase inhibition with aminoguanidine.

3 The response to histamine was significantly higher in the non-pregnant than in the pregnant women.

4 Histaminase inhibition increased the response to infused histamine significantly more in pregnant than in non-pregnant, women.

5 In pregnant, as well as in non-pregnant, women the increase in pulse rate during infusion of histamine was inversely proportional to the pulse rate at rest.

Acknowledgements

The statistical treatment was performed by fil. mag. Håkan Lindström. The study was aided by a grant from the "Medical Society of Göteborg

REFERENCES

Ahlmark A Acta physiol scandinav 9 suppl 28, 1944
Alles G A J Pharmacol Exp Ther 28 251 1926
Bader R A Bader M E Rose D J and Braunwald, E., J clin Invest 34 1524 1955
Best C H J J Physiol 67 256 1929
Bjurö T Lindberg, S and Westling, H Acta obst et gynec scandinav 43 201 1964
Brownlee K A Statistical Theory and Methodology in Science and Engineering, New York, 1961
Brown D D Tomchick R and Axelrod J J biol Chem 234 2948 1949
Clark D H and Tankel, H I Lancet 2, 886, 1954
G[illegible] G Lindell S-E and Westling, H unpublished
Janowitz H and Grossman M I Amer J Physiol 157 94 1959
Kahlson G Rosengren Elsa and Westling, H J Physiol 143 91 1958
Kullander S Acta endocrin 10 135 1952
Lieber E and Smith G B Chem Rev 25 213, 1939

crease in urinary histamine during human pregnancy The increase, however was small compared with that in the pregnant rat. Furthermore aminoguanidine given orally did not increase the urinary histamine measurably in the pregnant woman. *Michell* (1956) on the contrary found an increase in urinary histamine excretion after injecting histamine subcutaneously to healthy men when aminoguanidine in sufficiently high doses was given orally It should be emphasized that the present study for obvious ethical reasons was undertaken on women in relatively early pregnancy It seems probable that the observed effects of the aminoguanidine might have been larger in the later stages of pregnancy when histaminase activity is higher It is also possible that the aminoguanidine then would have had an effect on the basal pulse rate since endogenous histamine production may be higher at a later stage of pregnancy (*Kahlson et al* 1958 *Bjurö et al* 1964 *Granerus Lindell* and *Westling*, unpublished)

The increase in histamine sensitivity after histaminase inhibition demonstrated in the pregnant women in the present investigation might seem too small to represent an almost complete inhibition of histaminase If the results are compared with earlier studies on the histamine sensitivity of guinea pigs there is a certain resemblance *Lindell* and *Westling* (1954) studied the histamine sensitivity of the bronchi and the urinary bladder in guinea pigs before and after histaminase inhibition and *Westling* (1956 and 1957) studied the potentiation of histamine effects on the respiration and the rectal temperature in guinea pigs under the same conditions. In these investigations the potentiation of the histamine effects was mainly characterized by a prolongation of the response after inhibition of histaminase The maximal increase in sensitivity was about two-fold. As the guinea pig is very sensitive to histamine the authors considered the potentiation of the histamine response to be small. Even if the potentiation of the histamine effect in the present investigation was small, it was of statistical significance and suggests that the enzyme histaminase is active *in vivo* in the pregnant woman

SUMMARY

1 The response to histamine, infused intravenously was studied in 32 pregnant and 16 non-pregnant women. The pulse rate was chosen as the parameter for the effect of the histamine

2. To investigate if the enzyme histaminase modified the histamine effect in pregnant women, the response to infused histamine was also studied after specific histaminase inhibition with aminoguanidine.

3 The response to histamine was significantly higher in the non-pregnant than in the pregnant women.

4 Histaminase inhibition increased the response to infused histamine significantly more in pregnant than in non-pregnant, women.

5 In pregnant, as well as in non-pregnant, women the increase in pulse rate during infusion of histamine was inversely proportional to the pulse rate at rest.

Acknowledgements

The statistical treatment was performed by fil. mag Håkan Lindström The study was aided by a grant from the Medical Society of Göteborg

REFERENCES

Ahlmark A Acta physiol scandinav 9 suppl 28 1944

Alles, G A J Pharmacol Exp Ther 28 251 1926

Bader R A Bader M E Rose D J and *Braunwald*, E., J clin. Invest. 34 1524 1955

Best C H J J Physiol. 67 256 1929

Brandt T Lindberg, S and *Westling*, H Acta obst. et gynec. scandinav 43, 206 1964

Brownlee K A Statistical Theory and Methodology in Science and Engineering, New York, 1961

Brown D D Tomchick R and *Axelrod, J* J biol Chem. 234 2948 1949

Clark D H and *Tenkel H I* Lancet 2 896, 1954

Gravenius G Lindell S-E and Westling, H unpublished

Jenouert H and Grossman M I Amer J Physiol. 157 94 1959

Kahlson G Rosengren Elsa and *Westling, H* J. Physiol. 143 91 1958

Kullander S Acta endocrin 10 135 1952

Lieber E and Smith G B Chem. Rev 25 213, 1939

crease in urinary histamine during human pregnancy The increase however was small compared with that in the pregnant rat. Furthermore aminoguanidine given orally did not increase the urinary histamine measurably in the pregnant woman. *Michell* (*1956*) on the contrary found an increase in urinary histamine excretion after injecting histamine subcutaneously to healthy men when aminoguanidine in sufficiently high doses was given orally It should be emphasized that the present study for obvious ethical reasons was undertaken on women in relatively early pregnancy It seems probable that the observed effects of the aminoguanidine might have been larger in the later stages of pregnancy when histaminase activity is higher It is also possible that the aminoguanidine then would have had an effect on the basal pulse rate since endogenous histamine production may be higher at a later stage of pregnancy (*Kahlson et al* 1958 *Bjurö et al* 1964 *Granerus Lindell* and *Westling,* unpublished)

The increase in histamine sensitivity after histaminase inhibition demonstrated in the pregnant women in the present investigation might seem too small to represent an almost complete inhibition of histaminase If the results are compared with earlier studies on the histamine sensitivity of guinea pigs there is a certain resemblance *Lindell* and *Westling* (1954) studied the histamine sensitivity of the bronchi and the urinary bladder in guinea pigs before and after histaminase inhibition and *Westling* (1956 and 1957) studied the potentiation of histamine effects on the respiration and the rectal temperature in guinea pigs under the same conditions In these investigations the potentiation of the histamine effects was mainly characterized by a prolongation of the response after inhibition of histaminase The maximal increase in sensitivity was about two-fold. As the guinea pig is very sensitive to histamine the authors considered the potentiation of the histamine response to be small. Even if the potentiation of the histamine effect in the present investigation was small it was of statistical significance and suggests that the enzyme histaminase is active *in vivo* in the pregnant woman

SUMMARY

1 The response to histamine infused intravenously was studied in 32 pregnant and 16 non-pregnant women. The pulse rate was chosen as the parameter for the effect of the histamine.

2. To investigate if the enzyme histaminase modified the histamine effect in pregnant women the response to infused histamine was also studied after specific histaminase inhibition with aminoguanidine.

3 The response to histamine was significantly higher in the non-pregnant than in the pregnant women.

4 Histaminase inhibition increased the response to infused histamine significantly more in pregnant than in non-pregnant, women.

5 In pregnant, as well as in non-pregnant, women the increase in pulse rate during infusion of histamine was inversely proportional to the pulse rate at rest.

Acknowledgements

The statistical treatment was performed by fil. mag. Håkan Lindström. The study was aided by a grant from the "Medical Society of Göteborg

REFERENCES

Ahlmark A Acta physiol. scandinav 9 suppl 28 1944
Alles G A J Pharmacol. Exp. Ther 28 251 1926
Bader R A., Bader M. E. Rose D. J and Braunwald, E., J clin. Invest. 34 1524 1955
Best C H J J Physiol. 67 256 1929
Bjurö T Lindberg, S and Westling, H Acta obst. et gynec. scandinav 43 206 1964
Brownlee K A. Statistical Theory and Methodology in Science and Engineering, New York, 1961
Brown D D Tomchick R and Axelrod J J biol. Chem 234 2948, 1949
Clark D H and Tankel H I Lancet 2 886, 1954
Gronenca G Lindell S-E and Westling H unpublished
Janowitz H and Grossman, M I Amer J. Physiol. 157 94 1959
Kahlson G Rosengren Elsa and Westling, H J Physiol. 143 91 1958
Kullander S Acta endocrin 10 135 1952
Lieber E and Smith G B Chem. Rev 25 213, 1939

Lindahl Kerstin Lindell S E. Nilsson K. Schayer R. W., and *Westling, H* Arkiv f kemi *13* 379 1958
Lindahl Kiessling, Kerstin Ph. D thesis Uppsala, 1960
Lindberg, S Acta obst. et gynec. scandinav *42* suppl. 1 3 1963
Lindberg, S Lindell S -E. and *Westling, H* Acta obst. et gynec. scandinav *42* suppl. 1 35 1963
Lindberg, S M. D thesis Göteborg 1963
Lindberg, S and *Törnqvist A.* Acta obst. et gynec. scandinav 45 131 1966
Lindell S E. and *Westling, H.*, Acta physiol. scandinav 2 230 1954
Lindell S -E Nilsson K. Roos B.-E and *Westling, H.*, Brit. J Pharmacol *15* 351 1960
Marcou I Athanasiu-Vergu E Chiriceanu D., Cosma G Gingold N and *Parhon C -C* Presse Méd. *46* 371 1938
Michell R. G Brit. J Pharmacol. *11* 467 1956
Schayer R. W., Davis K. J and *Smiley R. L.* Amer J Physiol. 182 54 1955
Schayer R. W and *Cooper J A. D* J appl. Physiol. *9* 481 1956
Schayer R. W Amer J Physiol. *187* 63 1956
Schayer R. W Physiol. Rev *39* 116 1959
Schuler W Experientia *8* 230 1952
Swanberg, H Acta physiol. scandinav *23* suppl. 79 1950
Törnqvist A. unpublished
Weiss S Robb G P and *Ellis L. B.* Arch. intern. Med. *49* 360 1932
Westling, H., Acta physiol. scandinav *38* 91 1956
– Acta physiol. scandinav *39* 313 1957
Wicksell F Acta physiol. scandinav *17* 359 1949 a
– Acta physiol. scandinav *17* 359 1949 b
Widlund, G Acta obst. et gynec. scandinav 25 suppl. 1 1945

Received on May 19 1967

Pulkkinen, Martti O and Willman, Kalle Acta obst et gynec. scandinav 47 273, 1968

From the Department of Obstetrics and Gynaecology (*Professor L. Rauramo*) *and the Department of Physiology* (*Professor K. Hartiala*) *University of Turku Turku Finland*

ENZYMES AND ISOENZYMES IN MATERNAL AND FOETAL SERA

A study on lactate and isocitrate dehydrogenases, alkaline phosphatases and β-glucuronidase

BY

MARTTI O PULKKINEN AND KALLE WILLMAN

The placenta contains many enzymes in considerable amounts (*Hagerman* 1964) It is probable that these enzymes are produced by the placenta and are released into the maternal circulation during pregnancy On this basis many investigators have sought to associate such enzymes with placental function. Most of these studies have dealt with enzymes which are present in abundance in other body tissues and this explains why attempts to correlate serum enzyme levels with certain pathological states in obstetrics have not been entirely satisfactory To eliminate the influence of other tissues attempts have been made in recent years to determine only enzyme fractions which originate in the placenta. Thus for example Kitchner et al (1965) have determined the levels of alkaline phosphatases (AP) in maternal sera during pregnancy utilizing the greater thermal stability of those enzymes which originate in the placenta.

It has been found that the pH values and substrate concentrations at which the serum aspartate transaminase activity is at a maximum differ in liver and heart diseases (*Bergmeyer* 1962) The tissues of both these organs were found to produce the enzyme and release it into the blood. Similarly it might be possible that in pathological states the placenta secretes into the blood

enzyme fractions which differ from the enzymes normally present.

It is also known that different tissues contain different isoenzyme patterns of any given enzyme (*Wilkinson* 1965) and that a disease involving a certain organ causes a change in the isoenzyme distribution in the serum to one characteristic of the organ. Therefore it is possible that diseases of the placenta the foetus or the mother may alter the isoenzymes of the serum.

To study this latter hypothesis the levels of lactate dehydrogenase (LDH) β glucuronidase (β-G) isocitrate dehydrogenase (ICD) and alkaline phosphatase (AP) and those of the isoenzymes of LDH and AP in maternal and foetal serum were determined.

Particular attention was paid to the optimum conditions for the enzyme assays to the changes in the serum enzyme levels and to the distribution of isoenzymes in normal pregnancy and in pregnancy associated with disease in order to determine whether such changes can be used as diagnostic indices of maternal or foetal well being The correlation of the enzyme levels in maternal and foetal serum was also studied.

Material and Methods

Blood samples for enzyme assays were taken from 25 healthy non pregnant women between 22 and 40 years of age (mean age 27 years) from 434 pregnant women and from 258 umbilical cords The series of pregnant women comprised all patients admitted to the maternity wards whether in labor or not or treated at the out patient clinic of the Turku University Central Hospital during a period of three months The blood samples were taken in the morning from the cubital vein directly into a centrifuge tube with the exception of the patients suspected to delivery during the night of admission The blood was allowed to clot and immediately centrifuged. The sera were stored at +4 C until the enzyme assays were carried out. All the enzyme assays were performed within 24 hours of taking the samples. As control for AP isoenzyme studies sera from 11 men were collected.

Methods of enzyme assay

The total level of LDH was determined spectrophotometrically by the lactate-NAD method at pH 7.5 and +37° C. The pyruvate-NADH method was not used because endogenous pyruvate in the serum may have a variable inhibitory effect in the determination of various isoenzymes of LDH (*Thiers et al.* 1958). In addition, NADH is converted on storage into a compound that inhibits dehydrogenases (*King*, 1965). Substrate inhibition is not observed with lactate over wide range of concentration when the assay is carried out at 37° C (*Vesell* 1966).

The isoenzymes of LDH were separated from each other by disc electrophoresis on acrylamide gel (*Davis et al.* 1964) and measured by direct microdensitometry of the gel layer after staining with nitro blue tetrazolium (*Pulkkinen et al.* 1964).

The β-G levels were determined by the method of *Plaice* (1961) employing phenolphtalein glucuronide as substrate.

ICD assays. The pH at which ICD activity is maximal is generally considered to be 7.4 (*Bergmeyer* 1952). It was established in preliminary experiments that the enzyme activity was maximal at pH 7.5 (Tris buffer) when the serum was from non-pregnant woman but at a value of about 9 when the serum was obtained from pregnant woman, a placenta or an umbilical cord. Therefore pH 9.0 was employed in the subsequent assays. The substrate used was isocitrate and the coenzyme NADP. To reduce the incubation time and the required amount of serum, method was developed in which the reduced NADP was determined by fluorimetry (*Pulkkinen et al.* 1968).

AP assay. AP released from the placenta into the blood (*Boyer* 1961) has been stated to differ from AP normally present in blood by its greater thermal stability (*McMaster et al.* 1964). This has facilitated the determination of the AP released from the placenta during pregnancy. It was found in experiments with freshly prepared placental homogenates that about half of the enzyme activity was lost on keeping the serum at 65 C for 20 minutes and therefore this method could not be used.

The substrate chosen was β-glycerophosphate as the AP of the placenta has been found to have great affinity for this compound (*Anagnostopoulos et al.* 1958). In this respect the enzyme from the placenta differs from the AP found in other tissues and in normal serum. The pH of the medium was adjusted to 9.0 with glycine-sodium hydroxide buffer solution, as at this pH the activity of AP in sera from pregnant mothers and placentae was maximal.

Isoenzymes of AP were separated by disc electrophoresis (*Davis et al.* 1964) and stained using β-naphtyl phosphate as substrate. The liberated β-naphtol was tinted with Fast Blue B (*Sigma*) (*Allen et al.* 1963). No attempts were made to determine the isolated enzyme fractions quantitatively because of the poor intensity of the staining. The analytical procedure has been described in detail in previous paper (*Pulkkinen et al.* 1958).

Table I. Numbers of Enzyme Level Determinations on Maternal and Umbilical Cord Sera

Enzyme or Isoenzyme	Maternal Sera	Cord Sera
LDH	75	54
LDH isoenzymes	62	49
β-G	53	27
ICD	81	42
AP	93	54
AP isoenzymes	70	39

The levels of LDH ICD and AP are given below in thousandths of the *International Unit (m-I.U.)* and the levels of β-G in millionths of the International Unit (μ I.U.) The levels of isoenzymes of LDH are expressed as percentages of the total LDH content

All the determinations were performed in duplicate. The enzyme levels in one or two serum samples from non-pregnant women were determined daily whenever possible throughout the period of investigation and the same laboratory technician always performed all the assays of any one enzyme The numbers of enzyme level determinations performed on sera obtained from healthy pregnant women and from umbilical cords are given in Table I and the numbers of enzyme level determinations in various disease states in Table II The enzyme levels in sera from non-pregnant women from healthy pregnant women and from umbilical cords are shown in Tables III–VI.

The results of the enzyme assays were compared with the following factors age of the mother parity AB0 and rhesus blood groups of the mother and the child haemoglobin content of the blood of the mother (after delivery) weight of the newborn child Apgar score sex of child, weight of placenta duration of labor up to the time of sampling, total duration of labor and any maternal disease (*Table II*)

Results

Total LDH levels The results shown in Table III indicate that the total LDH level is higher in pregnant women at term than in non pregnant women ($p < 0.01$) The total LDH level in all the women pregnant for the third or more time is also significantly higher than in primigravidae ($p < 0.05$) In four patients with chronic hypertension the total LDH level was 192 ± 31.6 m I U

Table II Number of Enzyme Level Determinations on Maternal and Umbilical Cord Sera Various Pathological Conditions

	LDH		LDH-Iso-enzymes		β-G		ICD		AP		AP Iso-enzymes	
	m		m		m		m	c	m		m	c
Oedema	30	24	21	14	16	7	29	14	29	22	21	15
Blood pressure equal to or greater than 140/90	22	16	19	14	6	2	18	7	29	17	24	10
Proteinuria	6	3	4	3	2	1	5	3	8	2	7	1
Pre-eclampsia	18	11	16	10	5	4	11	5	21	14	18	6
Chronic hypertension	4	4	2	2*	1		5		9	6	8	5
Glucosuria	2	2	2		6	4	5	1	4	1	5	1
Pyelonephritis during pregnancy	15	13	10	11	6	5	5	9	9	3	0	3
Recurrent jaundice of pregnancy					3	1	3	1	4	2	2	2
Rh-immunization			2		4	1	1		7	4	4	2
Amniotic fluid containing meconium	10	8*	7	7	4	3	7	5	10	10	4	3
Premature deliveries	3	3		4	2	2	2	2	2	2	–	
Postmature deliveries	3	2*	2	1		1	2	2			1	1
Pathological neonatal jaundice	4	3	3	2	2	2	1	1	8	4	5	3
Apgar score 8 or less	7	5	9	6	3	3	7	6	10	7	0	5

Level deviating from those in normal pregnancies.
m = mother umbilical cord

Table III *Total LDH Levels in Sera of Non-Pregnant Women with Normal Pregnancies and in Serum from Umbilical Cord Blood*

Group	LDH		n
	M	S.E.	
Healthy non-pregnant women	103	± 4.6	17
Healthy pregnant women	134	± 9.3	42
All pregnant women.			
First pregnancy	127	± 7.5	36
Second pregnancy	134	± 13.2	22
Third pregnancy	167	± 9 8	9
Fourth or later pregnancy	177	± 24 1	8
Cord blood from normal pregnancies	257	± 14 4	28

Table IV *LDH-Isoenzyme Levels in Sera of Non-Pregnant Women Pregnant Women and Foetal Sera*

	LDH I		LDH II		LDH III		LDH IV		n
	M	S.E.	M	S.E.	M	S.E.	M	S.E.	
Healthy non-pregnant women	25 1	± 1.5	42.7	± 1 4	26.8	± 1 4	5.5	± 1.0	14
Healthy pregnant mothers	23.3	± 0 8	42.5	± 0.8	28.2	± 0 7	7.2	± 0.6	36
First pregnancy	22.8	± 0 7	42.1	± 1.3	29.8	± 0.9	8.1	± 0 7	27
Second pregnancy	22.7	± 1.2	43 0	± 1.3	27.6	± 1.3	8.5	± 1 1	21
Third pregnancy	24 8	± 1.8	40 1	± 1 0	28.8	± 1.5	6 6	± 1 0	7
Fourth or later pregnancy	28 8	± 1.9	41 4	± 1.2	25.3	± 1 4	5.3	± 0.8	6
Healthy foetuses	22.4	± 0.8	40.8	± 0.9	26 6	± 0 6	10.3	± 1 1	27

and in three with prolonged pregnancy 107 ± 24 0 m I U The umbilical cord serum LDH level was approximately twice as high as that in the sera of the women with normal pregnancies. The level in the cord serum was correlated with the placental weight ($r = -0\ 40$ $p < 0\ 01$ $n = 53$) In the women suffering from chronic hypertension the mean cord serum level was 331 ± 77 m I.U In 2 cases of prolonged pregnancy the cord enzyme levels were found to be 175 and 197 m I U The highest level 577 m I.U was found in the cord serum of an infant whose amniotic fluid was deep green.

LDH isoenzymes. Table IV shows that the relative content of the LDH fraction IV was higher in pregnant than in non-pregnant women ($p < 0.01$). The mean relative content of the LDH fraction I was higher in the sera of pregnant women who were in their fourth or subsequent pregnancy than in the sera of nulliparae ($p < 0.01$) or primiparae ($p < 0.05$). The relative content of the LDH fraction III was lower in the fourth or subsequent pregnancy than in the first ($p < 0.05$). No correlation was found between the levels of the LDH fractions and the total LDH levels in either the pregnant or non-pregnant women. The correlations between the levels of the LDH fractions I and III and between the levels of the fractions II and III in the sera of the mothers with normal pregnancies were significant ($r = -0.58$, $p < 0.001$ and $r = -0.40$, $p < 0.05$, $n = 33$). The mean relative content of the LDH fraction II was 40.2 ± 0.97 % ($n = 21$) in the pregnant mothers of blood group A and 44.8 ± 1.5 % ($n = 17$) in the pregnant mothers of blood group 0; the difference between these levels is significant ($p < 0.01$). In two mothers with chronic hypertension the relative contents of the LDH fraction I were 32.2 % and 28.6 %, those of the LDH fraction II 45.4 % and 39.7 %, those of the LDH fraction III 22.9 % and 22.5 % and those of the LDH fraction IV 5.1 % and 3.6 %. The mean relative content of the LDH fraction III was 31.1 ± 1.4 % in 10 mothers who had suffered from pyelonephritis at some stage of their pregnancy. This mean relative content is significantly higher than the mean relative content, 27.5 ± 0.6 %, in the other 47 mothers ($p < 0.05$).

Table IV reveals that the mean relative LDH IV content was higher in the cord serum than in the serum of both healthy pregnant and non pregnant women ($p < 0.01$). The correlations between the levels of the fractions LDH I and LDH III and those of the fractions LDH II and LDH IV in 25 normal cord sera were significant ($r = -0.68$, $p < 0.001$ and $r = -0.85$, $p < 0.001$). In two cord sera obtained from infants of women suffering from chronic hypertension the relative contents of the various LDH fractions were: LDH I 42.3 % and 28.6 %, LDH II 43.4 % and 36.8 %, LDH III 23.9 % and 11.5 %, LDH IV 8.8 % and 4.8 %.

Table V *Levels of β-Glucuronidase Isocitrate Dehydrogenase and Alkaline Phosphatase in Sera of Healthy Non-Pregnant Women Pregnant Women and Foetal Sera*

Group	β-G			ICD			AP		
	M	S.E.	n	M	S.E.	n	M	S.E.	n
Healthy non-pregnant women	129±	7 8	19	3 4±	0 4	13	8 4±	0 6	25
Healthy pregnant women	642±	55	33	7 4±	1.0	24	26 6±	2.1	52
Normal cord serum	223±	31	15	14.9±	1 7	19	19.5±	1 4	29

Sera taken during labour only

The mean relative content of fraction LDH II was lower 39 0±1 2 % in sera from 15 infants of blood group 0 than that from 10 infants of blood group B namely 42.6±0 86 % ($p < 0\ 05$) In two infants with dark green amniotic fluid the mean relative contents of the various LDH fractions were LDH I 43 0 % and 25 8 % LDH II 47 8 % and 32.6 % LDH III 21.3 % and 20 1 % LDH IV 4 9 % and 4 1 %

β-G Table V shows that the level of β-G is about 5 times as high in healthy pregnant women at term as in non pregnant women In three mothers suffering from recurrent jaundice of pregnancy the mean β-G level was 1517±448 μ I U The mean level of this enzyme in the cord serum was only about one third of that in the maternal serum

ICD The data in Table V reveal that the mean ICD level in healthy pregnant mothers who have uterine contractions was twice as high as the level in non pregnant women ($p < 0\ 001$) The ICD levels in the serum of pregnant women increased when uterine contractions occur ($r = 0\ 42$ $p < 0\ 01$ $n = 38$) Women at term but in whom no uterine contractions had occurred had a mean ICD level of 4.2±0 7 m I U ($n = 9$) which does not differ significantly from the mean level in non pregnant women. In pregnant women suffering from pre-eclampsia (mild or severe) the ICD levels were within the normal limits (8.5±1 6 $n = 11$) The highest mean ICD level, 26 1±12.8 m I U was found in

Table VI. *Number of AP Isoenzymes in Sera of Healthy Men, Women and Foetuses and in Sera of Pregnant Women with Various Diseases*

Group	No. of Subjects	Number of Sera Containing:		
		One Isoenzyme	Two Isoenzymes	Three Isoenzymes
Healthy non-pregnant women	23	7	12	4
Healthy men	11	3	5	3
Healthy pregnant women	59	41	10	8
Pregnant women with hypertension	24	19	4	1
Pre-eclamptic women	18	14	3	1
Pregnant women with pyelonephritis	9	7	2	0
Healthy foetuses	29	20	7	2

five mothers who had suffered from pyelonephritis during their pregnancy the highest level recorded in the study 76.4 m-I.U. was found in this group. The next highest level, 72.8 m-I.U., was recorded for a mother who had suffered from recurrent jaundice of pregnancy. The levels were normal in seven mothers whose amniotic fluids contained meconium.

The mean ICD level in cord blood serum of normal foetuses was approximately four times that in the serum of healthy non-pregnant women (Table V). The mean level in cord sera when the mothers had pre-eclampsia was 38.9 ± 8.8 m-I.U. (n = 5). High levels (mean 48.0 ± 12.9, p < 0.001, n = 7) were found when the mothers had suffered from pyelonephritis during their pregnancy the two highest levels 105 and 93.0 m-I.U. were recorded in this group. The levels were 57.0 and 93.0 m-I.U. in the cord bloods of two postmature infants and 39.4 and 49.5 m-I.U. in two asphyxiated infants. The mean level when the amniotic fluids contained meconium was 26.9 ± 8.1 m-I.U. (n = 5).

Total AP levels. The mean total AP level was about three times as high in normal pregnancy at term as in healthy non-pregnant women.

The mean AP level was 38.9 ± 10.1 m-I.U. in four pregnant

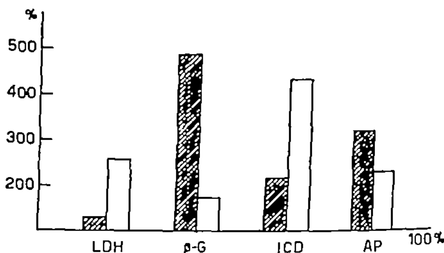


Fig 1 Excess enzyme levels in sera of healthy pregnant women (lined columns) and foetuses The base line gives the mean level of controls (as 100 per cent)

women with recurrent jaundice of pregnancy The mean AP level in the cord sera was about twice as high as the mean level in the sera of normal non pregnant women but significantly lower than the mean level in the pregnant women ($p < 0.05$) The highest AP level 47 0 m I U was found in the cord blood of an infant whose mother had suffered from recurrent jaundice of pregnancy and whose serum level was 66.5 m I U

AP isoenzymes The isoenzyme patterns in the various groups are shown in Table VI Only one isoenzyme was detected more frequently in pregnant women and their infants than in non-pregnant women and in men Five isoenzymes were found in the serum of a normal man. Up to seven bands were found in the isoenzyme patterns of several of the placentas studied.

General observations Factors that had no observable effect on the levels of various enzymes or their isoenzymes were the age of the pregnant women, the rhesus blood groups of the mother or child, the sex and maturity of the child the serum haemoglobin level of the mother after delivery and the duration of labor There was no correlation between the enzyme levels in the mothers and their infants (LDH $n = 45$ LDH isoenzymes

n = 35 β-G n = 23 AP n = 45) These enzyme levels are shown in Fig. 1 Parity or the AB0 blood groups of the mothers and their children were not found to be related to the levels of β-G ICD and AP enzymes and AP isoenzymes. The duration of contractions prior to sampling affected only the ICD level. The weight of the placenta was correlated only with the LDH level of the umbilical serum.

Discussion

It would be ideal if the state of the foetus and placenta could be followed during pregnancy by appropiate examination of the mother Possible the best chemical method at present available is to follow the excretion of hormones in the mother s urine (*e.g.* Klopper 1966)

Numerous studies have been reported in which enzyme levels in the serum of pregnant women suffering from various obstetric diseases have been determined (see *e.g.* Carol *et al.* 1965) but no method of enzyme determination has yet been universally adopted in clinical obstetric practice

The enzymes in the blood usually originate from several tissues As a certain enzyme may have different properties depend ing on the tissue from which it is derived (Wieland *et al.* 1963) it might be possible by choosing appropriate analytical conditions, to determine an enzyme originating in, for example, the placenta. In the present study it was found that the pH optimum of ICD in the serum of a pregnant woman and also in cord blood was clearly higher than that of the enzyme in the serum of a non pregnant woman. Because pregnancy itself does not cause an increase in the serum ICD level, it may be concluded that the organism strives to maintain a constant enzyme level by for example altering the production or release of enzymes from other tissues

A normal pregnancy usually brings about alterations in serum enzyme levels and these must be taken into account when hanges in enzyme levels arising from pathological conditions during pregnancy are being assessed. These physiologically elevat ed normal levels depend essentially on the method used to determine them and hence it is difficult to compare data reported

by different investigators *Meade et al* (1963) found elevated levels of LDH isoenzymes III and IV in pregnant women and assumed that they originated in the placenta where the levels of the isoenzymes III–IV are high (*Hawkins et al* 1966) *Hawkins et al.* (1966) found that the LDH I level rises in normal pregnancy but the LDH III and LDH IV levels rise during labor The results of the present study suggest that at least the LDH IV level increases during pregnancy This increase may be due to enzyme released from the placenta

It has already been established that the levels of the LDH isoenzymes II III and IV are higher in cord sera than in the sera of pregnant women (*Hawkins et al* 1966) We found only the level of LDH isoenzyme IV to be higher in the former sera The observed high total LDH level in the cord serum compared to that in the mother's serum has been reported earlier (*Lapan et al* 1959)

The level of β-G in the organism is regulated by hormones. In women oestrogenic hormones raise the β-G level in the liver (*Fishman* 1961) and blood (*Kasdon et al.* 1960) Oral contraceptives also produce a similar increase in the level of this enzyme in the blood (*Pulkkinen et al* 1968) The high β-G levels during pregnancy may hence be induced by hormones and may be due to enzyme released from the liver This is supported by the observation that the β-G level remains high in a woman post partum when stilboestrol is administered (*Fishman* 1950) In the present study levels of β-G in cord sera were considerably higher than those reported by *Kasdon et al* who found that they did not differ from serum levels in non-pregnant women. These latter workers found the mean β-G level to be three times higher in the sera of women in the third trimester of pregnancy than in the sera of non pregnant women, whereas the level was found to be about five times higher in the present study

Divergent results have been obtained in studies of the ICD levels in the sera of normal pregnant women (*Carol et al* 1965) Both normal (*Little et al* 1962) or elevated ICD levels (*Dawkins et al* 1961) have been found in women at labor Our results indicate elevated ICD levels at labor These high levels may be due to enzyme released from the placenta (*Dawkins et al* 1959)

The AP level has been found to increase during pregnancy (*Carol et al.* 1965) On the basis of electrophoretic (*Boyer* 1961) immunological (*Birkett* 1964) and heat inactivation studies (*McMaster et al.* 1964) it has been suggested that this is due to increased production of AP in the placenta.

Beckman et al. (1966) found by starch electrophoresis that only one AP fraction usually exists in the sera of healthy women. Sometimes however another unusual fraction is also found, which is genetically associated with AB0 blood groups. Using acrylamide gel electrophoresis we separated 1-3 AP fractions from most normal sera, although 5 AP fractions were found in the serum of a man. One or two additional AP fractions have been isolated from sera of pregnant women these fractions differ among different races and are related to the isoenzymes found in the placenta (Boyer 1963) Other investigators have stated that only one AP fraction occurs in the sera of women at term and during labor and that this fraction does not originate in the placenta but in bone marrow (*Meade et al.*, 1963) In our study we found that one fraction occurs more often in the sera of preg nant women than in the sera of non-pregnant women. As we found simultaneously as many as seven different AP isoenzymes in placenta it seems that all fractions do not pass from the placenta into the maternal circulation. *Beckman et al.* (1965) found only one AP isoenzyme in 98 per cent of the cord sera they studied and a band similar to the pregnancy band in the mother s sera in two per cent. We frequently found 2 to 3 isoenzyme bands in the patterns of cord sera. It is obvious that the previously reported divergent results on the occurrence of AP isoenzymes in sera in different conditions may be due to the use of different electrophoretic procedures, and hence no definite onclusions can be drawn about the occurrence and inheritance of AP isoenzymes.

As far as we are aware no enzyme has yet been found the lev ls of which in the blood of healthy mothers and their foetuses are correlated (*Lapan et al* 1959) Our own findings reveal that no such correlation exists between the levels of the enzymes we ha e studied Neither was such a correlation established between the levels of the LDH and AP isoenzymes in the mother and

by different investigators *Meade et al.* (1963) found elevated levels of LDH isoenzymes III and IV in pregnant women and assumed that they originated in the placenta where the levels of the isoenzymes III–IV are high (*Hawkins et al* 1966) *Hawkins et al* (1966) found that the LDH I level rises in normal pregnancy but the LDH III and LDH IV levels rise during labor The results of the present study suggest that at least the LDH IV level increases during pregnancy This increase may be due to enzyme released from the placenta.

It has already been established that the levels of the LDH isoenzymes II III and IV are higher in cord sera than in the sera of pregnant women (*Hawkins et al* 1966) We found only the level of LDH isoenzyme IV to be higher in the former sera. The observed high total LDH level in the cord serum compared to that in the mother s serum has been reported earlier (*Lapan et al* 1959)

The level of β-G in the organism is regulated by hormones. In women oestrogenic hormones raise the β G level in the liver (*Fishman* 1961) and blood (*Kasdon et al* 1960) Oral contraceptives also produce a similar increase in the level of this enzyme in the blood (*Pulkkinen et al* 1968) The high β-G levels during pregnancy may hence be induced by hormones and may be due to enzyme released from the liver This is supported by the observation that the β G level remains high in a woman post partum when stilboestrol is administered (*Fishman* 1950) In the present study levels of β-G in cord sera were considerably higher than those reported by *Kasdon et al* who found that they did not differ from serum levels in non-pregnant women. These latter workers found the mean β-G level to be three times higher in the sera of women in the third trimester of pregnancy than in the sera of non pregnant women whereas the level was found to be about five times higher in the present study

Divergent results have been obtained in studies of the ICD levels in the sera of normal pregnant women (*Carol et al* 1965) Both normal (*Little et al* 1962) or elevated ICD levels (*Dawkins et al* 1961) have been found in women at labor Our results indicate elevated ICD levels at labor These high levels may be due to enzyme released from the placenta (*Dawkins et al* 1959)

Normal β-G and AP levels have been observed in pregnant women with high blood pressure (*Odell et al.*, 1948 *Carol et al* 1965 *Mukherjee* 1951) Chronic hypertension in the mother led to elevated total LDH levels in the sera of the mothers and their offsprings. In both groups of sera the LDH I level was high and the LDH III level low The negative correlation between the levels of LDH I and LDH III was thus evident in this disease. The high levels of LDH I in the blood in pathological conditions may hence be due to increased release of this fraction from the kidneys where it is present in abundance (*Wieland et al.* 1963)

Increased total LDH levels have been found in the urines of patients with pyelonephritis (*Nánró et al.* 1966) High levels of LDH III were found in the sera of such patients in this study It is of interest to note that the total serum LDH remains unchanged in both pregnant and non-pregnant women with this disease (*Kerppola et al* 1959) The simultaneous high ICD level in the cord serum may be a sign not only of changes in the placenta but also of the effect of maternal toxins on the foetus.

Asphyxia was observed to effect changes in the enzyme levels of the foetal sera only High LDH (LDH I) and ICD levels were found in the sera of infants whose amniotic fluid contained meconium and who had low Apgar scores. These levels are also high in the cord sera of postmature infants Despite our attempts to develop appropriate methods we did not succeed in finding any changes in the levels of enzymes in the maternal sera that would be of alue in the diagnosis of asphyxia in the child.

Recurrent jaundice of pregnancy has been found to be associated with high levels of certain enzymes (*Ikonen* 1964) According to our results the levels of ICD and β-G seem to be good indicators of the development of recurrent jaundice and its abatement n pregnant women

The levels of enzymes in pregnant women studied to date are of importance in differential diagnosis in only a few obstetric onditions such as ecurrent jaundice of pregnancy Altered enzyme levels are found in the sera of infants of mothers who ha suffered from arious obstetric diseases but blood samples naturally cannot be obtained at the desirable time On the other hand, when a change in serum enzyme level has been found in

foetus This independence of the enzyme levels in the mother and foetus is indicated also by the observed large difference in these levels The levels of LDH and ICD were higher in the cord sera, but the levels of β-G and AP higher in the maternal sera (Fig. 1)

We did not find in the literature any observations that would indicate that the levels of different LDH isoenzymes in sera are correlated. We did not find any correlation between the levels of these isoenzymes in the sera of non pregnant women but did find that the levels of these enzymes in the sera of healthy pregnant women and in the cord sera were closely correlated. The level of the LDH isoenzyme III varied inversely to the levels of the LDH isoenzymes I and II in the maternal sera. The organism may maintain the total LDH level constant by changing the relative proportions of the LDH isoenzymes. No one isoenzyme level was found to be correlated with the total LDH level and hence did not alone decisively determine the total LDH level.

The observed correlation between the total LDH level in the umbilical cord serum and the weight of the placenta may be taken to indicate that the enzyme is partly derived from the placenta The high LDH IV levels in the cord sera support this conclusion because this isoenzyme mainly occurs in the placenta (*Hawkins et al* 1966)

When the effect of various physiological factors on the levels of enzymes was onsidered it was observed that the total LDH level increased with the number of pregnancies evidently because of an increase in the content of the LDH I isoenzyme This may reflect altered adaptation of the organism after each pregnancy

Higher than normal levels of LDH (*Kubli* 1961) β-G (*Odell et al* 1948) ICD (*Little et al* 1962) and AP (*Mukherjee* 1951) have been reported in the sera of pregnant women suffering from pre-eclampsia. However normal levels have also been found in pregnar women with this disease of LDH (*Hawkins et al.* 1966) G (*Pasetto et al* 1956) and AP (*Kubli* 1961) In our series high ICD levels were found in the sera of two of eleven pre-eclamptic women and also in cord serum in three of five cases. These latter results are in accordance with those of *Pelu son* (1964) and show that a study of the ICD levels in pregnant women with pre-eclampsia is of little value

suffering from chronic hypertension than in the sera of healthy pregnant women. High ICD levels were sometimes found in sera of pregnant women suffering from pre-eclampsia. Changes in enzyme levels were observed in cord sera when the mothers had chronic hypertension and the LDH and ICD levels were higher in these sera when the mothers suffered from pre-eclampsia or pyelonephritis. Asphyxia and prolonged pregnancy led to marked changes in the levels of LDH and its isoenzymes and in the ICD levels in the cord sera. When the mother had experienced recurrent jaundice of pregnancy high levels of β-G ICD and AP were found in the maternal sera. No correlation was found to exist between the levels of enzymes and isoenzymes in the maternal and foetal sera when the pregnancies developed normally but such correlations may exist in pathological conditions.

Acknowledgements

The authors are indebted to Mrs. Jäkälä for the technical assistance in enzyme analyses and to all clinical nurses especially to Miss Niemi and Mrs Tervo who helped in the collection of the serum samples. Mr Löppönen B A., computerized the results.

Abbreviations

LDH	lactate dehydrogenase (E C 1 1 1 27)
β-G	β-glucuronidase (E C 3 2 1 31)
ICD	isocitrate dehydrogenase (E C 1 1 1 41)
AP	alkaline phosphatase (E C 3 1 3 1)
M	mean
NAD	nicotinamide adenine dinucleotide
NADP	nicotinamide adenine dinucleotidephosphat
	number of determinations
P	risk level
	correlation coefficient
S E	standard error of the mean
m-I U	one thousandth of the International Unit (E C)
μ-I U	one millionth of the International Unit (E.C.)
m	mother
	umbilical cord

the mother a similar change may be observable also in the foetus. It seems that although there are no correlations between enzyme levels in the sera of the healthy mothers and their infants such correlations may exist in pathological conditions The explanation for this correlation could be the same reason for the elevation of enzyme activities both in maternal and foetal sera (*e g* toxins in pyelonephritis) or the changes of placental permeability through damage (*e g* pre-eclampsia, chronic hypertension, postmaturity) Conversely when affected solely foetus behaves independently For that purpose see Table II

The enzyme and isoenzyme levels in the sera of pregnant women must be concluded to be of only limited value in the assessment of the conditions of the mother and foetus.

SUMMARY

The levels of lactate dehydrogenase (LDH) β-glucuronidase (β G) isocitrate dehydrogenase (ICD) and alkaline phosphatase (AP) and those of the LDH and AP isoenzymes were estimated by analyzing serum samples from 25 healthy non pregnant women 434 pregnant women and 258 samples from umbilical cords Acrylamide disc electrophoresis was used for isoenzyme determinations The pH at which the ICD of the placenta exhibited peak activity was exceptionally high (pH 9) The AP of the placenta was found to be heat labile The total levels of the enzymes studied were with the exception of ICD high during pregnancy the ICD levels were elevated only when contractions occurred. The LDH and ICD levels were twice as high in the cord sera as in the sera of mothers but the levels of β-G and AP were lower in the former than in the latter sera. Both in the maternal and cord sera a correlation was found between some LDH isoenzymes The mean LDH IV isoenzyme levels were higher in the maternal and cord sera than in the sera of non-pregnant women. Only one AP isoenzyme was present more often in the sera of pregnant women than in the sera of non pregnant women.

The LDH levels were higher in the sera of pregnant women

suffering from chronic hypertension than in the sera of healthy pregnant women. High ICD levels were sometimes found in sera of pregnant women suffering from pre-eclampsia. Changes in enzyme levels were observed in cord sera when the mothers had chronic hypertension and the LDH and ICD levels were higher in these sera when the mothers suffered from pre-eclampsia or pyelonephritis. Asphyxia and prolonged pregnancy led to marked changes in the levels of LDH and its isoenzymes and in the ICD levels in the cord sera. When the mother had experienced recurrent jaundice of pregnancy high levels of β-G ICD and AP were found in the maternal sera. No correlation was found to exist between the levels of enzymes and isoenzymes in the maternal and foetal sera when the pregnancies developed normally but such correlations may exist in pathological conditions.

Acknowledgements

The authors are indebted to Mrs. Jäkälä for the technical assistance in enzyme analyses and to all clinical nurses especially to Miss Niemi and Mrs Tervo who helped in the collection of the serum samples. Mr Lyppönen, B.A., computerized the results.

Abbreviations

LDH	lactate dehydrogenase (E C 1 1 1 27)
β-G	β-glucuronidase (E C 3 2 1 31)
ICD	isocitrate dehydrogenase (E C 1 1 1 41)
AP	= alkaline phosphatase (E C 3.1 3 1)
M	mean
NAD	nicotinamide adenine dinucleotide
NADP	= nicotinamide adenine dinucleotidephosphate
	number of determinations
P	risk level
	correlation coefficient
S E	standard error of the mean
mI U	one thousandth of the International Unit (E.C)
μI U	one millionth of the International Unit (E.C.)
m	mother
	umbilical cord

the mother a similar change may be observable also in the foetus. It seems that although there are no correlations between enzyme levels in the sera of the healthy mothers and their infants, such correlations may exist in pathological conditions. The explanation for this correlation could be the same reason for the elevation of enzyme activities both in maternal and foetal sera (*e.g.* toxins in pyelonephritis) or the changes of placental permeability through damage (*e.g.* pre-eclampsia, chronic hypertension, postmaturity). Conversely, when affected solely, foetus behaves independently. For that purpose see Table II.

The enzyme and isoenzyme levels in the sera of pregnant women must be concluded to be of only limited value in the assessment of the conditions of the mother and foetus.

SUMMARY

The levels of lactate dehydrogenase (LDH), β-glucuronidase (β-G), isocitrate dehydrogenase (ICD) and alkaline phosphatase (AP) and those of the LDH and AP isoenzymes were estimated by analyzing serum samples from 25 healthy non-pregnant women, 434 pregnant women and 258 samples from umbilical cords. Acrylamide disc electrophoresis was used for isoenzyme determinations. The pH at which the ICD of the placenta exhibited peak activity was exceptionally high (pH 9). The AP of the placenta was found to be heat labile. The total levels of the enzymes studied were, with the exception of ICD, high during pregnancy; the ICD levels were elevated only when contractions occurred. The LDH and ICD levels were twice as high in the cord sera as in the sera of mothers, but the levels of β-G and AP were lower in the former than in the latter sera. Both in the maternal and cord sera a correlation was found between some LDH isoenzymes. The mean LDH IV isoenzyme levels were higher in the maternal and cord sera than in the sera of non-pregnant women. Only one AP isoenzyme was present more often in the sera of pregnant women than in the sera of non-pregnant women.

The LDH levels were higher in the sera of pregnant women

Thiers R. E. and *Vallee* B. L. Ann N Y Acad. Sci 75 214 1958
Vesell E. S. Nature 210 421 1966
Wieland T and *Pfleiderer* G (Multiple Formen von Enzymen) Advanc. Enzymol. 25 329 1963
Wilkinson J H Isoenzymes Spon Ltd., London, 1965

Received on June 13 1967

REFERENCES

Allen J M and *Hyncik G* Histochem. Cytochem. *11* 169 1963

Anognostopoulos C and *Matsudaria H* Int. Symp on Enzyme Chemistry Tokyo-Kyoto 166 1958

Beckman L Björling G and *Christodoulou C* Acta genet. (Basel) *16* 59 1966

Beckman L and *Grirea M* Acta genet. (Basel) *15* 218 1955

Bergmeyer H V Methoden der enzymatischen Analyse Verlag Chemie, GmbH Weinheim pp 701 and 984 1962

Birkett D J Thesis Sydney 1964

Boyer S H Science *134* 1002, 1961

— Ann. N Y Acad Sci. *103* 938 1963

Carol W and *Bonow A.* Zbl Gynäk. *87* 426 1965

Davis B J and *Ornstein L.* Ann N Y Acad Sci. *121* 305 1964

Dawkins M J R MacGregor W G and *McLean A E M* Lancet 2 827 1959

Dawkins M J R and *Wigglesworth J S* J Obstet. Gynaec Brit. Cwlth. 68 264 1961

Fishman W H Chemistry of Drug Metabolism Springfield p. 127 1961

Hagerman Dw D Fed. Proc. Symp. *23* 85 1964

Hawkins D F and *Whyley G A.* Clin chim. Acta. *13* 713 1966

Ikonen E. Acta obst et gynec. scand. *43* Suppl 5 1964

Kasdon S C Romano P and *Hatzimichael A.* Obstet. and Gynec. *15* 367 1960

Kerppola W Nikkilä E. A. and *Pitkänen E* Acta Med. Scand. *164* 357 1959

King, J Practical Clinical Enzymology van Nostrand, London 1965

Kitchner P N Neale F C Posen S and *Brudenell-Woods J* Amer J Clin. Path *44* 654 1965

Klopper A. Research on Steroids *II* 63 1966

Kubli F Gynaecologia *151* 72 1961

Lapan B and *Friedman M M* J Lab Clin Med *54* 417 1959

Little W A. and *Kirpalani G* Amer J Obstet. Gynec *83* 1346 1962

M Master Y Tennant R Cl bb J S and *Neale F C* J Obstet. Gynaec Brit. Cwlth. *71* 735 1964

M ue B W and *Rosalki S B.* J Obstet Gynaec Brit. Cwlth *0* 852, 1963

Mukherjee Ch J Indian Med. Ass *21* 43 1951

Näntö V Kasanen A and *Lehtonen A.* Ann Med Int. Fenn. *23* 55 1966

Odell L. D and *McDonald D F* Amer J Obstet Gynec *56* 74 1948

Pasetto N and *Ermglia* Minerva Ginec *8* 133 1956

Pehrson S L. Acta obst. et gynec. scand. *43* 69 1964

Plaice C H J J Clin Path. *14* 661 19 1

Pulkkinen M O and *Willman K.* Acta obst. et gynec scand. *46* 525 1968

in normal pregnancy and in patients suffering from obstetric hepatosis.

Material and methods

The patients investigated comprised 20 healthy non-pregnant women of child-bearing age who served as controls 18 healthy pregnant women, 9 pregnant women suffering from obstetric hepatosis, and a 37 year-old pregnant woman with hepatic cirrhosis The mean age in the control group was 32 years in the healthy pregnant group 28 years and in the obstetric hepatosis group 25 years. The diagnosis of obstetric hepatosis was based on the usual clinical criteria such as general pruritus elevated total serum bilirubin and elevated transaminase (GOT GPT) and alkaline phosphatase values The pruritus disappeared and the laboratory tests reverted to normal after delivery in every case. None of the women in the control series were taking contraceptive pills.

The urine was collected during each 24-hour period, and was stored alkaline and protected from light. Porphobilinogen (PGB) and δ-aminolaevulic acid (ALA) determinations were carried out according to the method of *Mauzerall* and *Granick* (1956) The coproporphyrin isomers I and III were separated by means of thin-layer chromatography and determined quantitatively as described in detail by *Koskelo* and *Toivonen* (1966) The determinations in the healthy women were made during the first trimester in three cases during the second in three and during the third trimester in twelve cases In 15 cases the analysis was repeated from one to 15 weeks (average 3 weeks) post partum. In the obstetric hepatosis series control determinations were carried out in 7 cases on an average 6 weeks post partum.

Results

The results are shown in Table I and figures 1 and 2.

PBG.—The mean urinary output of PBG in the pregnant women did not significantly differ from that in the control group.

ALA.—Comparison of the mean urinary excretion of ALA in the control subjects with that in the healthy pregnant subjects and patients with obstetric hepatosis shows statistically highly

Koskelo, Pentti, and Toivonen, Ilkka, Acta obst et gynec, scandinav 47 292, 1968
From the Third Department of Medicine (Professor E. A. Nikkilä M.D.)
University of Helsinki Helsinki Finland

URINARY EXCRETION OF COPROPORPHYRIN ISOMERS I AND III AND δ AMINOLAEVULIC ACID IN NORMAL PREGNANCY AND OBSTETRIC HEPATOSIS

BY

PENTTI KOSKELO AND ILKKA TOIVONEN

Although slight deviations from the normal values of standard laboratory tests related to liver function, such as the bromsulphtalein, alkaline phosphatase and transaminase reactions are seen in a minority of normal pregnancies (*Christhilf* and *Bonsnes* 1950 *Friedman et al* 1961 *Ikonen* 1964) the accepted opinion has been that liver function is normal during pregnancy How ever there is now good evidence that this may not be the case (*Kappas* 1967) Among patients suffering from pruritus and jaundice in late pregnancy (obstetric hepatosis) the standard liver function tests are altered in the majority of cases (*Ikonen* 1964) pointing to a marked disturbance in the liver function

A clear deviation from the normal distribution of urinary coproporphyrin isomers I and III can be frequently found in various liver diseases the deviation being most marked and diagnostic in the Dubin Johnson syndrome (*Koskelo et al* 1967) A mild liver functional impairment due to oral contraceptives also results in a slight increase of urinary coproporphyrin isomer I and δ-aminolaevulic acid excretion (*Koskelo et al* 1966)

The purpose of this paper is to present data on the urinary excretion of porphyrin praecursors and coproporphyrin isomers

in normal pregnancy and in patients suffering from obstetric hepatosis.

Material and methods

The patients investigated comprised 20 healthy non-pregnant women of child-bearing age who served as controls 18 healthy pregnant women, 9 pregnant women suffering from obstetric hepatosis and a 37 year-old pregnant woman with hepatic cirrhosis The mean age in the control group was 32 years in the healthy pregnant group 28 years, and in the obstetric hepatosis group 25 years. The diagnosis of obstetric hepatosis was based on the usual clinical criteria such as general pruritus, elevated total serum bilirubin and elevated transaminase (GOT GPT) and alkaline phosphatase values. The pruritus disappeared and the laboratory tests reverted to normal after delivery in every case. None of the women in the control series were taking contraceptive pills.

The urine was collected during each 24-hour period, and was stored alkaline, and protected from light. Porphobilinogen (PCB) and δ-aminolaevulinic acid (ALA) determinations were carried out according to the method of *Mauzerall* and *Granick* (1956) The coproporphyrin isomers I and III were separated by means of thin-layer chromatography and determined quantitatively as described in detail by *Koskelo* and *Toivonen* (1966) The determinations in the healthy women were made during the first trimester in three cases, during the second in three and during the third trimester in twelve cases In 15 cases the analysis was repeated from one to 15 weeks (average 3 weeks) post partum. In the obstetric hepatosis series control determinations were carried out in 7 cases on an average 6 weeks post partum.

Results

The results are shown in Table I and figures 1 and 2.

PBG —The mean urinary output of PBG in the pregnant women did not significantly differ from that in the control group.

ALA —Comparison of the mean urinary excretion of ALA in the control subjects with that in the healthy pregnant subjects and patients with obstetric hepatosis shows statistically highly

Koskelo, Pentti, and Toivonen, Ilkka, Acta obst et gynec. scandinav 47 292, 1968
From the Third Department of Medicine (Professor E. A. Nikkilä M.D.)
University of Helsinki Helsinki Finland

URINARY EXCRETION OF COPROPORPHYRIN ISOMERS I AND III AND δ-AMINOLAEVULIC ACID IN NORMAL PREGNANCY AND OBSTETRIC HEPATOSIS

BY

PENTTI KOSKELO AND ILKKA TOIVONEN

Although slight deviations from the normal values of standard laboratory tests related to liver function such as the bromsulphtalein alkaline phosphatase and transaminase reactions are seen in a minority of normal pregnancies (*Christhilf* and *Bonsnes* 1950 *Friedman et al* 1961 *Ikonen* 1964) the accepted opinion has been that liver function is normal during pregnancy However there is now good evidence that this may not be the case (*Kappas* 1967) Among patients suffering from pruritus and jaundice in late pregnancy (obstetric hepatosis) the standard liver function tests are altered in the majority of cases (*Ikonen* 1964) pointing to a marked disturbance in the liver function.

A clear deviation from the normal distribution of urinary coproporphyrin isomers I and III can be frequently found in various liver diseases the deviation being most marked and diagnostic in the Dubin Johnson syndrome (*Koskelo et al* 1967) A mild liver functional impairment due to oral contraceptives also results in a slight increase of urinary coproporphyrin isomer *I* and δ-aminolaevulic acid excretion (*Koskelo et al* 1966)

The purpose of this paper is to present data on the urinary excretion of porphyrin praecursors and coproporphyrin isomers

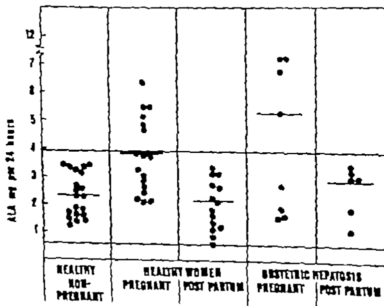


Fig. 1. Urinary excretion of δ-aminolaevulic acid (ALA) in the control and experimental series. The shaded area denotes the normal range.

tion did not significantly differ from that of the controls. In patients with obstetric hepatosis the urinary coproporphyrin excretion was above the upper limit of normal in every case. The mean excretion of coproporphyrin in this series was significantly higher than that of the control subjects and of the healthy pregnant subjects ($p < 0.001$). The total coproporphyrin excretion reverted to the normal range post partum in every case studied with the exception of the patient with cirrhosis, who excreted 193 g per 24 hrs.

A very marked deviation from the normal non pregnant urinary coproporphyrin isomer I and III distribution was seen in the majority of cases during normal pregnancy and in all cases with obstetric hepatosis. When the results are expressed as a percentage of isomer III of the total (Fig. 2) it can be seen that the isomer III content was below normal in 16 out of 18 healthy

Table I. *Urinary Excretion of δ-Aminolaevulic Acid (ALA) Porphobilinogen (PBG) and Coproporphyrin During Normal Pregnancy and Obstetric Hepatosis*

		ALA mg/24 hr	PBG mg/24 hr	Coproporphyrin	
				Isomer I ug/24 hr	Isomer III ug/24 hr
Healthy non-pregnant	Range	1 19–3.48	0 78–2.69	9–29	27 105
	Mean	2.25	1 66	14	57
	S D	0.82	0.57	5	20
Healthy pregnant	Range	2.07–6 45	0.82 3 65	13–91	23–66
	Mean	3 84	2.34	40	43
	S. D	1.37	0 82	23	13
Healthy women post partum	Range	0.57–4 68	0.88–2 87	7 39	15–80
	Mean	2.12	1 75	17	45
	S. D	1 09	0.58	9	21
Obstetric hepatosis pregnant	Range	1 69–12.70	1 12–2.74	86–222	45–111
	Mean	5.26	2.11	143	68
	S. D	3 70	0.55	51	18
Obstetric hepatosis post partum	Range	1 07–4 23	1 64–3.50	13–29	48–80
	Mean	2.83	2.35	20	61
	S. D	1 03	0 73	5	16

significant differences ($p < 0\ 001$) There is no statistically significant difference between the mean values in the two groups of pregnant patients The urinary ALA output was above the upper limit of normal in 7 out of 20 healthy pregnant women and in 5 out of 9 patients with obstetric hepatosis (normal range = mean ± S D) Increased ALA excretion was seen in three cases out of five studied during the first and second trimesters and in seven out of 13 studied during the last trimester ALA excretion reverted to normal after delivery in every in the healthy pregnant women studied and in six out of seven women with obstetric hepatosis In the patient with hepatic cirrhosis the ALA excretion was 4 68 mg per day during pregnancy and 0 61 mg 6 months post partum.

Coproporphyrin —A slight elevation above the normal (mean +2 S.D) in the total urinary coproporphyrin output was seen in three cases in the healthy pregnant group but the mean excre-

isomer distribution in normal pregnancy and in patients with obstetric hepatosis is mainly due to increase in the excretion of isomer I, the isomer III excretion remaining unchanged or being only slightly changed. A control examination performed post partum revealed a slight deviation from normal in one case only In one patient with cirrhosis the relative coproporphyrin III out put during the last trimester was only 18 per cent. Six months post partum the percentage of isomer III was 30 which is still well below normal.

Discussion

Normally coproporphyrin is excreted principally in the faeces because the normal healthy liver has such a great affinity and concentration power for it that the concentration in the blood never attains a level high enough to promote significant urinary excretion. When hepatic excretory dysfunction arises from any cause the plasma levels of coproporphyrin and porphyrinogen are elevated, leading to excretion of more in the urine and less in the faeces via bile (*Rimington* 1963) Earlier investigations (*Koskelo et al.* 1967) and the present observations indicate, that in many liver diseases it is the coproporphyrin isomer I excretion which rather sensitively shifts from biliary to urinary excretion, whilst in many cases the isomer III excretion remains unchanged. The phenomenon was noted during normal pregnancy in all cases examined in the last trimester In obstetric hepatosis the urinary isomer I excretion was on average higher than in normal preg nancy but there was some overlap of values. The abnormal coproporphyrin isomer I excretion during normal pregnancy is most probably the result of oestrogen-induced hepatic functional impairment The same phenomenon can be seen to a lesser degree in women taking oestrogen-containing steroid mixtures for contracepti e purposes (*Koskelo et al.* 1966) The altera tions seen in hepatic function during normal pregnancy are at tributed to biological effects of the exceptional quantities of oestradiol, oestriol and related compounds which are produced during normal pregnancy (*Kappas* 1967) For example the alteration in the storage capacity and maximal excretory rate of

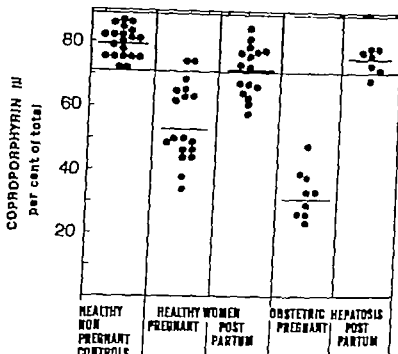


Fig. 2. Urinary coproporphyrin isomer distribution expressed as percentage of isomer III of the total in the control and experimental series. The shaded area denotes the normal range.

pregnant women. All cases studied during the last trimester exhibited a deviation from the normal isomer distribution. The difference between the mean in this group and that in the control group is statistically significant ($p < 0.001$). A control examination which was performed one to two weeks post partum revealed that there was still a slight deviation from the normal in six out of 15 cases studied post partum. There was however no statistically significant difference between this and the control group. In two cases with abnormal isomer deviation one week post partum, an analysis was made six months later and a normal isomer distribution was found.

In patients with obstetric hepatosis the relative urinary output of isomer III was on an average lower than in the healthy pregnant group. The difference is statistically significant ($p < 0.001$). It is evident that the deviation from the normal non pregnant

isomer distribution in normal pregnancy and in patients with obstetric hepatosis is mainly due to increase in the excretion of isomer I the isomer III excretion remaining unchanged or being only slightly changed. A control examination performed post partum revealed a slight deviation from normal in one case only In one patient with cirrhosis the relative coproporphyrin III out put during the last trimester was only 18 per cent. Six months post partum the percentage of isomer III was 30 which is still well below normal.

Discussion

Normally coproporphyrin is excreted principally in the faeces because the normal healthy liver has such a great affinity and concentration power for it that the concentration in the blood never attains a level high enough to promote significant urinary excretion. When hepatic excretory dysfunction arises from any cause the plasma levels of coproporphyrin and porphyrinogen are elevated, leading to excretion of more in the urine and less in the faeces via bile (*Rimington* 1963) Earlier investigations (*Koskelo et al.* 1967) and the present observations indicate that in many liver diseases it is the coproporphyrin isomer I excretion which rather sensitively shifts from biliary to urinary excretion, whilst in many cases the isomer III excretion remains unchanged. The phenomenon was noted during normal pregnancy in all cases examined in the last trimester In obstetric hepatosis the urinary isomer I excretion was on average higher than in normal pregnancy but there was some overlap of values. The abnormal coproporphyrin isomer I excretion during normal pregnancy is most probably the result of oestrogen-induced hepatic functional impairment. The same phenomenon can be seen to a lesser degree in women taking oestrogen-containing steroid mixtures for contraceptive purposes (*Koskelo et al.* 1966) The altera tions seen in hepatic function during normal pregnancy are at tributed to biological effects of the exceptional quantities of oestradiol oestriol and related compounds which are produced during normal pregnancy (*Kappas* 1967) For example the alteration in the storage capacity and maximal excretory rate of

BSP during normal pregnancy reflects disturbances in two independent hepatic mechanisms (*Combes et al* 1963)

The increased urinary excretion of ALA found during the latter part of normal pregnancy and more frequently in patients with obstetric hepatosis may also be a result of an increase in the circulating oestrogens Whether the increased excretion of ALA is a result of a change in its excretion route or whether it reflects its increased synthesis is not known. The latter alternative seems plausible since it is known that sex steroids act as inducers of the synthesis of aminolaevulic acid synthetase in chick embryo liver cells in vitro (*Granick* 1966)

SUMMARY

Urinary excretion of porphobilinogen, δ-aminolaevulic acid and coproporphyrin isomers I and III was studied in 18 healthy pregnant women and 9 patients suffering from obstetric hepatosis The mean excretion of δ-aminolaevulic acid was significantly higher both in the healthy pregnant women and in those with hepatosis as compared with a control group of 20 healthy non pregnant women. There was no significant difference in the mean excretions of porphobilinogen between the three groups The total mean urinary coproporphyrin output was normal in uncomplicated pregnancy but was significantly elevated in all patients with obstetric hepatosis. There was a significant deviation from the normal urinary coproporphyrin isomer I and III distribution during normal pregnancy and in obstetric hepatosis. This was mainly due to an increased excretion of coproporphyrin isomer I which was significantly higher in association with obstetric hepatosis than during normal pregnancy

Acknowledgements

Our thanks are due to Tapani Luukkainen M D and to Ensio Ikonen, M D for delivering the urine samples and allowing us to study the records of the patients which were hospitalized in the I and II clinics of Obstetrics and Gynaecology University of Helsinki This study was supported by grants from the Signe and Ane Gyllenberg foundation Helsinki and the Finnish Medical Research Council

REFERENCES

Christhilf S. M., and Boessner R. W Am. J. Obst. & Gynec. 59 1100 1950
Combes B., Shibata, H., Adams R., Mitchell B D and Trammel, V J Clin. Invest. 42 1431 1963
Friedman M M. Lapan B. and Taylor T H Am. J. Obst. & Gynec. 82, 132, 1961
Granick S J Biol. Chem. 241 1359 1966
Ikonen, E., Acta obst. et gynec. scandinav 43 suppl. 5 1954
Kappas A., Gastroenterology 52 113, 1967
Koskelo P Eisalo A., and Toivonen I., Brit. Med. J 1 652, 1966
Koskelo P Toivonen I and Adlercreutz H Clin Chem. 13 1005 1967
Koskelo P and Toivonen I Scand. J clin. Lab. Invest. 18 543, 1966
Mauzerall, D and Granick S J Biol. Chem 219 435 1956
Rimington C., S Afr J Lab. Clin Med. 9 255 1963

Received on Oct 6 1967

Jonasson, Lars Erik and Persson, Bengt H. Acta obst. et gynec. scandinav. 47 300 1968
From the Department of Obstetrics and Gynaecology (Professor C. Gemzell)
University Hospital Uppsala Sweden

SPECTROPHOTOMETRIC SCREENING OF AMNIOTIC FLUID IN HAEMOLYTIC DISEASE AND HEPATOSIS GRAVIDARUM

BY

LARS ERIK JONASSON AND BENGT H. PERSSON

In the early nineteen fifties *Bevis* (1950 1952, 1953 and 1956) demonstrated the presence of blood pigments in the amniotic fluid obtained by transabdominal amniocentesis He showed that spectrophotometric scanning of the amniotic fluid permitted an accurate assessment of the severity of the haemolytic process in cases of Rh iso-immunization. Since then a number of reports have confirmed the clinical importance of this observation (*Walker* 1957 *Liley* 1961 1963 and 1965 *Mackay* 1961 *Freda* 1962, 1965 *Fairweather* and *Walker* 1964)

The severity of the haemolytic disease in the foetus may be predicted by quantitative evaluation of the yellow pigmentation in the amniotic fluid This is based upon the presence of a characteristic spectral absorption curve with a peak at 450 m*u* In amniotic fluid from normal pregnancies at term the absorption curve is approximately linear from 365 to 600 m*u* *Liley* (1961) provided an index for the practical application of the measurement of the aberrant peak and demonstrated the importance of observing the behaviour of the peak with the passage of gestation time in order to predict the state of the foetus in utero and the ultimate prognosis The severity of the haemolysis is reflected approximately by the height of the peak which may reach a value indicative of imminent intra uterine death. The majority of predictions made in this way are reliable but the existence of errors must be acknowledged in order to make a proper evalua

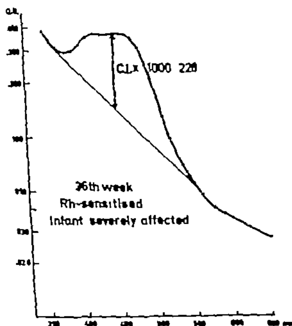


Fig 1 Colour index in Rh-sensitized case with severely affected infant showing cord haemoglobin value of 3 8 g % but no hydrops

tion of Liley s technique Some of the pitfalls like contamination of the specimen with blood pigments of maternal or foetal origin, staining of the amniotic fluid with meconium or turbidity due to contaminating cellular debris or lipid drops are readily recogniz ed Others like the presence of methaemalbumin and oxy haemoglobin, may disturb the quantitative estimation of the peak at 450 m (*Fleming* and *Woolf* 1965) Apparently the peak is formed by the presence of bilirubin or bilirubinoid pigment in amniotic fluid but the precise biochemical state of the chromogen is unknown and the possibility of a varying mixture of pigments or biochemical states of the same pigment remains (*Liley* 1963) *Fleming* and *Woolf* (1965) proposed a quantitative estimation of the concentration of bilirubin in amniotic fluid by spectrophotometry at certain predicted wave-lengths. The result from

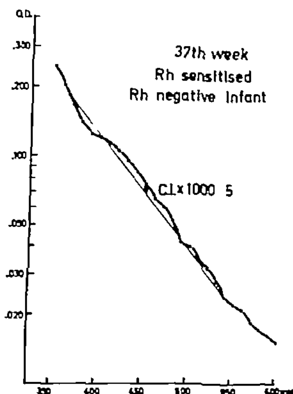


Fig 1 b Colour index in a Rh-sensitized case with a healthy Rh-negative infant

these measurements is said to be reproducible to within narrow limits and the recovery of added bilirubin is satisfactory

For some years we have analysed liquor amnii routinely as a guide in the management of Rh sensitized patients During that time we found several cases with high colour index (C.I.) though Rh iso-immunization was absent. All the cases with falsely high values showed signs of hepatosis gravidarum mostly without any icterus. Obviously the abnormally high peak at 450 m in these cases reflected the increased bilirubin content of the maternal blood and not haemolysis of the foetal blood. Hepatosis gravidarum is by no means a rarity in our population, the frequences ranging between 0.3 and 0.5 per cent of delivered in patients (about 3400 deliveries a year) The real incidence is certainly higher as minor cases are easily overlooked

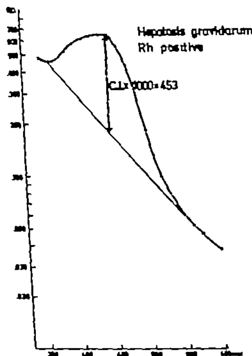


Fig 1 Extremely high colour index in Rh-positive woman at the 31st week of pregnancy showing typical hepatosis gravidarum (clinically subicteric)

or treated as out patients. A large majority of the cases of hepatosis gravidarum are anicteric. It has been shown by *Arfwedson* (1953 1956) *Thorling* (1955) *Ljunggren* (1956) *Svanborg* and Olsson (1959) and *Ikonen* (1964) that serum bilirubin is elevated and liver function tests show signs of impairment in women with pruritus gravidarum, which thus is a low-degree manifestation of hepatosis gravidarum. Small series of cases have also been published by *Moore* (1963) and *Brown et al.* (1963)

The present report includes amniotic fluid analyses from Rh-sensitized pregnancies, ABO-iso-immunized pregnancies, Rh-positi pregnancies at various age of gestation as well as 14 cases of hepatosis gravidarum. The erroneous effect of increased

maternal bilirubin on the interpretation of the spectrophotometric analyses of amniotic liquor is established.

Materials and Methods

A total of 107 patients was studied This series included sensitized cases Rh-positive cases (normal patients) and 14 cases of pregnancy hepatosis The women with hepatosis were arbitrarily chosen from all those treated in the clinic during one year All but one of them were Rh positive and none of them showed any evidence of infectious hepatitis One of the patients with hepatosis was Rh-negative and sensitized. In two of the cases the pregnancies terminated spontaneously in the 32nd week and one of the children succumbed to immaturity and postnatal asphyxia. The other 11 pregnancies were otherwise uneventful and ended with deliveries of healthy children at term

The amniocenteses were performed at various times of gestation. A four inch needle was used for uterine puncture No special effort was made to localize the placenta except for certain cases of severe haemolytic disease The puncture was performed on the ventral side of the foetal chest just caudal to the foetal head. About 20 ml of amniotic fluid was aspirated and the specimens were immediately placed in clean glass tubes and protected from exposure to light. Amniotic fluid from normal cases at term was obtained by vaginal amniocentesis. Within the next few minutes the fluid was centrifuged for about 20 minutes at 4500 r p.m After filtration the supernatant fluid was analysed in a Beckman B spectrophotometer measuring the absorption curve from 350 up to 700 m at certain predicted intervals (Fig 1)

The colour index (C I)was estimated according to *Liley* by measuring the height of the peak at 450 m In certain cases the bilirubin concentration in liquor amnii was calculated according to the method of *Fleming* and *Woolf* (1965)

The haemoglobin value as well as the concentration of serum bilirubin of the child was determined from cord blood immediately after birth Maternal serum bilirubin content as well as serum transaminases (SGOT SGPT) and alkaline phosphatase were

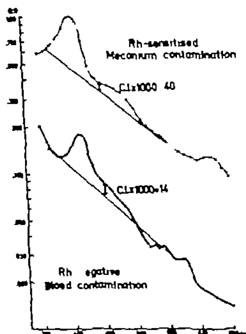


Fig. 1 d. Effect of contamination with meconium and blood on the pattern of the spectrophotometric curve. In both cases the infants were healthy at birth.

determined routinely in order to recognize cases of pregnancy hepatosis in the material.

The amniotic fluid samples were further studied with additional techniques by one of us (L.E.J.) the results of which will be reported separately.

The clinical material is compiled in Table I where the cases are grouped according to their clinical type.

Results

Thirteen out of 139 amniocenteses yielded blood-contaminated fluid, which in 5 samples made a reliable measurement impossible in spite of repeated centrifugation and filtration. In each of these cases a living child was subsequently delivered. The first attempt

maternal bilirubin on the interpretation of the spectrophotometric analyses of amniotic liquor is established.

Materials and Methods

A total of 107 patients was studied. This series included sensitized cases Rh positive cases (normal patients) and 14 cases of preg nancy hepatosis The women with hepatosis were arbitrarily chosen from all those treated in the clinic during one year All but one of them were Rh positive and none of them showed any evidence of infectious hepatitis. One of the patients with hepatosis was Rh negative and sensitized. In two of the cases the pregnancies terminated spontaneously in the 32nd week and one of the children succumbed to immaturity and postnatal asphyxia. The other 11 pregnancies were otherwise uneventful and ended with deliveries of healthy children at term.

The amniocenteses were performed at various times of gestation A four inch needle was used for uterine puncture. No special effort was made to localize the placenta except for certain cases of severe haemolytic disease. The puncture was performed on the ventral side of the foetal chest, just caudal to the foetal head. About 20 ml of amniotic fluid was aspirated and the specimens were immediately placed in clean glass tubes and protected from exposure to light. Amniotic fluid from normal cases at term was obtained by vaginal amniocentesis. Within the next few minutes the fluid was centrifuged for about 20 minutes at 4500 r p.m After filtration the supernatant fluid was analysed in a Beckman B spectrophotometer measuring the absorption curve from 350 up to 700 mμ at certain predicted intervals (Fig 1)

The colour index (C I)was estimated according to *Liley* by measuring the height of the peak at 450 mμ In certain cases the bilirubin concentration in liquor amnii was calculated according to the method of *Fleming* and *Woolf* (1965)

The haemoglobin value as well as the concentration of serum bilirubin of the child was determined from cord blood immediately after birth Maternal serum bilirubin content as well as serum transaminases (SGOT SGPT) and alkaline phosphatase were

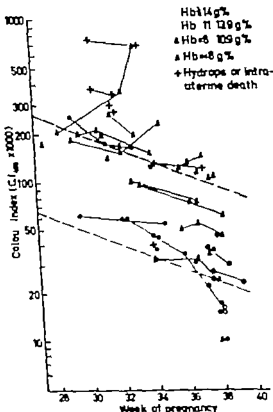


Fig. 2 a Distribution of cases of Rh-sensitized, Coombs-positive pregnant women with respect to colour index of amniotic fluid and haemoglobin level of cord blood

at amniocentesis failed to yield any fluid in 5 cases. No clinical complications were noted in connection with the amniocenteses except for one case of premature separation of the placenta during delivery In this case the foetus died during delivery but autopsy did not reveal whether the foetus succumbed to intra uterine asphyxia or to erythroblastosis. The overall results in the Rh immunized cases are plotted in Fig. 2 a where the colour indices are grouped according to *Liley* (1961) Fig. 2 b shows the cases of hepatosis and the control cases plotted in the same manner

Table I *Clinical Material*

Type of Cases	Number of Cases	Number of Liquor Analyses	Number of Living Children	Number of Blood Exchanges	Perinatal Deaths	
					Number	Cause of Death
Rh-immunized (direct Coombs pos.)	61	78	53	114/51	8	Abruptio plac (1) Erythroblastosis (4) Diabetes + erythroblastosis (1) Erythroblastosis + immaturity (1) Heart disease (1)
Rh immunized (direct Coombs neg.)	14	26	13	1/1	1	Birth defect
ABO-immunized	5	5	5	0	–	–
Hepatosis gravidarum	14	17	13	5/1	1	Immaturity + RDS
Normal patients	13	13	13	0	–	–
Total	107	139	97	120	10	

Case 14 a–c (see Table II)

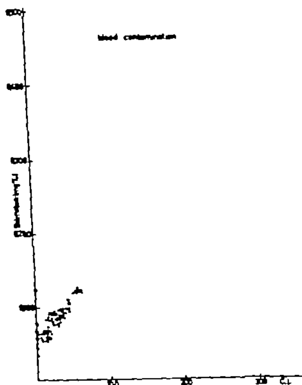


Fig 3 Correlation between colour index acc to *Liley* and bilirubin content calculated acc to *Fleming* and *Woolf*

the amniotic fluid. All the Rh-negative infants as well as all Rh-positi e infants who did not need exchange transfusion showed a Hb/Bil quotient above 5 In all these cases the amniotic fluid showed a colour index below 40 The infants who succumbed to erythroblastosis showed a very low Hb/Bil. quotient (below 2) and a C.I above 110 The middle zone (C.I >40 <110) included Rh-positive infants who needed one or more exchange trans fusions. This classification of the clinical material is principally in accordance with that of Liley when applied to pregnancies of 32 to 37 weeks.

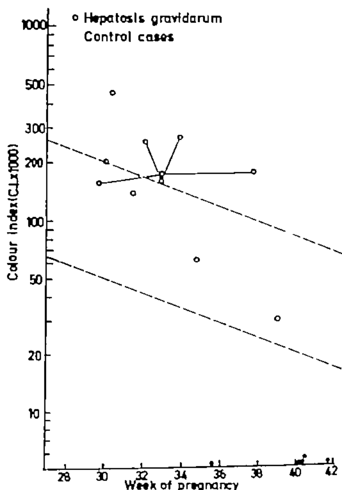


Fig. 2 b Distribution of colour indices in 14 cases of hepatosis gravidarum as compared with 16 healthy patients.

In a trial to predict the cord haemoglobin from the colour index of the amniotic fluid following *Liley*s (1963) criteria we found a correct prediction in 55 % of the Rh-sensitized cases in the present series (disregarding blood-contaminated samples) This figure is lower than that reported by *Liley* and too low to be useful in clinical practice

After delivery the quotient between haemoglobin level of the cord blood and bilirubin concentration of cord serum was cal culated and correlated with the colour index found prenatally in

of these dangerous cases the maternal serum bilirubin showed practically normal values and only two of the women showed a very slight icterus. Thus in a Rh-sensitized woman the presence of subclinical hepatosis may provoke an erroneous prediction of the severity of the haemolytic disease and accordingly an unneeded premature obstetrical interference.

Case number 14 (Table II) is an example of the combined effect of Rh-sensitization and hepatosis. A 23 year old secundigravida with no iso-immunization during her first pregnancy was admitted because of D-immunization with a final titre of 1 1280 (papain method) and 1 160 (indirect Coombs test) The first amniocentesis showed so high a C.I. (287) that an intrauterine foetal transfusion was considered. Therefore an intraamniotic injection of 20 millilitres of 60 % Urografin was given the day before the second amniocentesis and this may explain the lower C.I of 178 It was then observed that the patient had somewhat elevated values of SGOT (64) and SGPT (116) and the transfusion was postponed. Instead Caesarean section was performed in the 34th week after the third amniocentesis which yielded meconium stained fluid with a C.I. of 290 The 2800 g child was quite active and survived. It was not hydropic but required 5 exchange transfusions. Capillary haemoglobin at birth was 11.9 g % and cord bilirubin was 6.2 mg %

According to our previous experience colour indices of this height would mean impending intrauterine death. This case thus illustrates the difficulties introduced by even a slight hepatosis upon the evaluation of the degree of Rh-sensitization.

Discussion

The assessment of haemolytic disease from amniotic fluid analyses as devised by *Liley* has been generally accepted as a valuable parameter in chosing the optimal time for delivery Our experience with the method is in accordance with this view and we have found it an indispensable complement to the estimation of maternal antibody titres. Our view is that it is most valuable in cases of severe iso-immunization, whereas in cases of slight iso-

Table II. *Cases of Hepatosis Gravidarum*

Case Number	Week of Pregnancy	Maternal Serum Bilirubin (Total)	Maternal Alkaline Phosphatase	Maternal SGOT	Maternal SGPT	C.I.X 1 000	Calculated Bilirubin Content (F W) (mg/100 ml)
1 a	31	1.2	8 0	25	53	155	-
1 b	38	1.9	11 7	42	59	180	0 275
2	31	5 1	13.2	14	7	453	
3	31	4 1	4 7	62	51	200	0.302
4	32	2.1	4 4	105	190	138	-
5	35	1 1	22.0	360	320	62	0.134
6	38	4 4	5 4	540	600	159	0.265
7	39	1 1	8 7	23	34	0	0.062
8	40	1 0	10 7	275	375	35	0.097
9	40	0 6	5 4	92	95	24	0.082
10	40	1 0	5 4	145	380	59	0 058
11	41	3 7	10 0	225	270	190	0.320
12	41	1 1	9 7	117	180	64	0 158
13	42	0 4	3 7	108	190	120	0.230
14 a	32	0 8	3.3	55	75	287	0 449
14 b	33	0.9	4.2	64	116	178	0.285
14 c	34	0.9	4 1	52	9[illegible]	290	0.543

Hepatosis combined with Rh-sensitization

The correlation between the colour index and the corresponding bilirubin values of amniotic fluid calculated according to *Fleming* and *Woolf* (1965) is seen in Fig 3

As shown in the figure there is a strong positive correlation between the calculated bilirubin content and the colour index of the liquor specimens tested Accordingly the simple estimation of C.I in the majority of cases gives as good information as the more complicated calculation proposed by *Fleming* and *Woolf* The latter may be preferred when gross contamination with blood pigments is encountered in the test sample

The figures found in cases of hepatosis gravidarum are detailed in Table II.

As shown in this table the colour index is very high in 8 out of 14 cases reaching the immediate danger zone of Liley In four

the foetus general condition and prognosis especially in cases with severe foetal affection.

Hepatosis raises the extinction values of amniotic fluid in the same way as does Rh-sensitization with haemolytic disease of the foetus and may thus confuse the evaluation of amniotic fluid analysis.

Among a total of 126 abdominal amniotic punctures there occurred one foetal death which might possibly be due to the amniocentesis. In 5 cases gross blood contamination made reliable colour index estimation impossible. In these cases spectrophotometric estimation of the bilirubin content might be of some value otherwise it does not yield any further information.

REFERENCES

Arfwedson H Sv Läkartidn 50 1685 1953
Obst Gyn 7 274 1956
Bevis D D A Lancet 2 443 1950
Lancet 1 395 1952
J Obst & Gyn Brit Emp. 60 244 1953
J Obst & Gyn Brit Emp 63 68 1956
Brown D F *Porte* E A and *Reder* J Arch. intern. Med. 111 592, 1963
Fairweather D V I and *Walker* W J Obst. & Gyn. Brit. Comm. 71 48, 1964
Fleming A F and *Woolf* A J Clin Chim Acta 12 67 1965
Freda V J Am J Obst & Gyn. 84 1756 1962
Am J Obst & Gyn 92 341 1965
Ikonen E Acta obst et gyn scand. 43 Suppl 5 1964
Liley A W Am J Obst & Gyn. 82 1359 1961
Am J Obst & Gyn 86 485 1963
Pediatrics 35 836 1965
Ljunggren G Nord Med. 55 373 1956
Mackay E V Aust New Zeal J Obst Gyn. 1 78 1961
Moore H C Lancet 2 57 1963
Svanborg A and *Olsson* S Am J Med 27 40 1959
Thorling L Acta Med Scand 151 Suppl 302, 1955
Walker A H C Brit Med J 2 376 1957

Received on June 17 1967

immunization it does not permit any differentiation between affected and unaffected foetuses. This is however of little practical importance as the prognosis in these cases is good with proper postnatal management. In the present series the cord haemoglobin value shows *only a poor correlation* with the colour index of the amniotic fluid. Nevertheless the colour index gives a good picture of the general condition and the prognosis of the foetus In cases of heterozygous husbands serial amniotic fluid analyses seem to be of great use to detect Rh negative foetuses. Apparently no additional information could be gained by determining the bilirubin value by the method of *Fleming* and *Woolf* except for the cases where gross contamination of the amniotic fluid by blood disturbs the colour index determination. By determining the spectrophotometric absorption values at close intervals it is possible to detect the interference of contaminating blood pigments which give a deformed curve

In cases of hepatosis gravidarum we have found spectrophotometric curves which could not be distinguished from curves obtained from severe cases of haemolytic disease Thus hepatosis gravidarum would present difficulties if it occurs in a Rh immunized patient. Therefore it is recommended that *liver function* tests be performed routinely in all Rh immunized cases to exclude interference by maternal bilirubin transferred to the amniotic fluid So far we have not been able to reveal any significant difference between the physico-chemical state of the amniotic bilirubin in cases of hepatosis and in haemolytic disease Investigations on this special problem are in progress and will be reported separately

SUMMARY

Clinical experience of spectrophotometric analysis of amniotic fluid is reported in 76 cases of Rh sensitization. A standard method has been used to group the extinction peak values Fourteen cases of hepatosis and 13 non-sensitized patients have also been studied, using the same technique

The method used has been found to be of definite clinical value in cases of Rh sensitization. It makes possible a fair judgment of

the foetus general condition and prognosis, especially in cases with severe foetal affection.

Hepatosis raises the extinction values of amniotic fluid in the same way as does Rh-sensitization with haemolytic disease of the foetus and may thus confuse the evaluation of amniotic fluid analysis.

Among a total of 126 abdominal amniotic punctures there occurred one foetal death which might possibly be due to the amniocentesis. In 5 cases gross blood contamination made reliable colour index estimation impossible. In these cases spectrophotometric estimation of the bilirubin content might be of some value, otherwise it does not yield any further information.

REFERENCES

Arfwedson H Sv Läkartidn 50 1685 1953
Obst Gyn 7 274 1956
Bevis D D A Lancet 2 443 1950
Lancet 1 395 1952
J Obst & Gyn Brit Emp 60 244 1953
J Obst & Gyn Brit Emp 63 68 1956
Brown D F *Porta* E A and *Reder* J Arch. Intern. Med. *111* 592, 1963
Fairweather D V I and *Walker* W J Obst & Gyn Brit Comm. *71* 48 1934
Fleming, A F and *Woolf* A J Clin Chim Acta 12, 67 1965
Freda V J Am J Obst & Gyn *84* 1756 1962
Am J Obst & Gyn 92 341 1965
Ilonen E Acta obst et gyn scand 43 Suppl. 5 1964
Liley A W Am J Obst & Gyn 82 1359 1961
Am J Obst & Gyn 86 485 1963
Pediatrics 35 836 1965
Ljunggren G Nord Med. 55 373 1956
Mackay E V Aust New Zeal J Obst Gyn 1 78 1961
Moore H C Lancet 2 57 1963
Stenborg A and *Olsson* S Am J Med. 27 40 1959
Thorling L Acta Med Scand 151 Suppl. 302, 1955
Walker A H C Brit Med J 2, 376 1957

Received on June 17 1967

Naver Erik, Acta obst. gynec. scandinav 47 314, 1968

From the Department of Gynaecology and Obstetrics (Halfdan Lefèvre M.D and Jan Weber M D) The Glostrup Hospital Glostrup Denmark

THE INCOMPETENT CERVIX AND ITS TREATMENT IN HABITUAL ABORTION AND PREMATURE LABOUR

BY

ERIK NAVER

The incompetent cervix syndrome refers to a functional or anatomical defect which results in deficient closure of the internal cervical os during advancing pregnancy This leads to a premature termination of an otherwise normal pregnancy in the form of late abortion or premature labour

Since the pioneer studies of *Palmer* and *Lacomme* (1948) *Lash* and *Lash* (1950) and *Shirodkar* (1955) this condition has been the subject of thorough investigations The numerous publications on aetiology diagnosis and treatment have established the incompetent cervix syndrome as a well-defined clinical entity and as the aetiological factor in a group of habitual, late abortions and premature deliveries which may be successfully treated by surgery

Concerning the pathogenesis various views have been expressed in respect to the defective mechanism.

Danforth (1947) believed that the junction of the upper muscular and the lower fibrous part of the lower segment formed the barrier which kept the foetus *in utero* as the uterine cavity gradually expanded due to the growth of the conceptus and the increased intrauterine pressure

Youssef (1958) and *Mann et al* (1961) attribute to the isthmic area a sphincteric function whose insufficiency plays a role in the mechanism of abortion.

Asplund (1952) and *Borell* and *Fernström* (1953) by radiographic studies demonstrated that a functional sphincter controls

the width of the isthmus under the influence of hormonal variations, oestrogenic hormone relaxing and progesterone contracting the sphincter

It seems to be agreed that it is the isthmic area which forms the closing mechanism and that various aetiological factors may cause defective closure at this site.

As the most important aetiological factor of cervical incompetence previous authors have mentioned traumatic damage during previous delivery especially in precipitate delivery with an excessively large infant and obstetrical manoeuvres such as extraction (*McDonald* 1957 *Lash* A F 1960)

But often there is a history of an apparently normal delivery and successive pregnancies may end in earlier and earlier delivery gradually as the cervix is weakened by each episode of obstetric trauma (*Baden* and *Baden* 1960)

The injury may also be due to surgery i.e. it may be a poorly healed scar left by cervical Caesarean section, forced dilatation of the cervical canal by Hegar s dilators in the treatment of abortion or in curettage or in the treatment of dysmenorrhoea. Amputation and tears of the cervix may lead to the same damage if the region around the isthmus is involved.

Lash (1960) emphasized the prophylactic value of avoiding forced dilatation by Hegar s dilators of an atraumatic delivery of the head and of the use of antibiotics in the event of surgical procedures on the cervix and where Caesarean section is performed after drainage of the amniotic fluid.

However there are also cases in which it is impossible to demonstrate an anatomical defect due to previous trauma and in which the isthmic defect must be considered *functional*.

Danforth et al (1960) pointed out not only the histology of the cervix but also the histochemical changes in its wall which result in softening and dilatation. They demonstrated that changes in the connective-tissue ground substance and in the arrangement of the fibrils make the cervix lose its tense structure and yield to the increased intrauterine pressure. However we have no explanation of what starts the otherwise normal course of events prematurely

Finally there are cases of cervical incompetence in which the

aetiological factor is believed to be *congenital malformation* of the cervix, consisting of a preponderance of muscular tissue over fibrous structures which normally predominate instead of the reverse, which is normally the case (*Roddick et al.* 1961)

But whether the defect is traumatic, functional, or congenital, the clinical picture is the same

For many years it has been known that among habitual abortions there is a specific group characterized by painless, passive dilatation of the cervix in the second trimester leading to rupture of the membranes and expulsion of an immature foetus.

This sequence has a tendency to recur in consecutive pregnancies so that a probable diagnosis can usually be made if a patient has an obstetric history of this nature

This form of habitual abortion usually occurs during the 16th–20th weeks of pregnancy In such cases the cervix undergoes changes which normally do not take place until the time of delivery Softening, dilatation and loss of tonus, but without demonstrable uterine contractions

This process may take 6–8 days during this period and before the cervical effacement has become complete there is passage of clear watery discharge from the vagina and the patient feels pressure behind the symphysis.

Concurrently the isthmic-sphincteric function is lost, resulting in prolapse of the membranes through the dilated os Uterine contractions appear and the ovum is expelled, either *in toto* or after rupture of the membranes in the course of one or a very few contractions

The condition is rare *Barter et al* (1958) found an incidence of 1 in 1842 deliveries *Raphael* (1966) 1 in 1930 deliveries and *Quigley* and *Lynes* (1964) 0 21 per cent of deliveries and 0 6 per cent of all gynaecological admissions

In spite of this quantitatively modest proportion of incompetent cervices among the causes of premature delivery the syndrome is of interest since by the recognition and correct treatment of the condition it is possible to combat effectively one of the causes of premature delivery Thereby one aspect of prematurity which still remains one of the unsolved obstetrical problems, may be countered.

From the diagnostic point of view an obstetric history of 3 or more consecutive late abortions or premature deliveries of a typical course is an almost definite diagnostic criterion of incompetent cervix (*Shirodkar* 1955 *Lash* 1964 *Barter et al.* 1958)

When the patient is not pregnant, the cervix may be of an entirely normal appearance.

A possible defect in the cervical canal may be palpated immediately after abortion (*Lash* and *Lash* 1950) Moreover it may be a sign of incompetent cervix if a No. 6 (or larger) Hegar s dilator can pass the internal os without resistance in the latter half of the menstrual cycle.

Lastly radiography may demonstrate too wide a cervical canal (*Mann* 1959 *Rubovits et al.* 1953 *Asplund* 1952)

Since however apparently normal cervices may become incompetent while apparently defective cervices may maintain the conceptus it is generally agreed that these procedures are not of absolute diagnostic significance. The only entirely certain diagnostic criterion of incompetent cervix is the observation during pregnancy that the cervix gradually becomes effaced and dilates with bulging of the amniotic sac but without simultaneous contractions or bleeding (Baden and *Baden* 1960)

T eatment

The principle of the therapeutic methods is to strengthen the incompetent cervix and thereby re-establish its resistance to the increased intrauterine pressure during advancing pregnancy

This is fulfilled by two types of surgical procedures

1 Cervical repair as advocated by Lash and *Lash* (1950) which is carried out in the non-pregnant state and consists in an excision of a segment of the anterior cervical wall on a level with the internal os and closure with catgut in two layers.
2 The cerclage method as advocated by *Shirodkar* (1955) which may also be performed during pregnancy and which i technically simpler It is applicable *in all forms* of cervical incompetence—regardless of the aetiology—while cervical

repair can be used only in cases where an anatomical defect is demonstrable

Another objection to the cervical excision is that in some cases it leaves a postoperative sterility problem (*Quigley* and *Lynes* 1964) and that the diagnostic problems during the non-pregnant state are not definitely solved (*Rovinsky* 1961)

The cerclage method which appears to eliminate these problems has become the method of choice. In the course of time several modifications of the original Shirodkar method have been made, both in respect to suture material and technique

Instead of the originally employed homologous strip from the fascia lata unabsorbable, synthetic material is being used now

Barter et al (1958) use a Dacron tape applied subepithelially by the method of *Shirodkar* The tape is applied with an aneurysm needle, around the cervix subepithelially after incision of the skin of the cervix, anteriorly and posteriorly and tied on a level with the internal os It is further fastened to the cervix by silk sutures anteriorly and posteriorly

Ritter and *Ritter* (1961) modified the Shirodkar operation by incising the epithelium all the way around the cervix and placing the suture under the cardinal and uterosacral ligaments

McDonald (1957) has described a simpler suturing method, applying the cerclage as a purse string suture of silk or mersilene on the ectocervix at the junction of the fornix and cervical epithelium.

Lastly *Benson* and *Durfee* (1965) have advocated a trans abdominal cerclage method during pregnancy in cases where the vaginal approach is contra indicated or technically out of the question *e g* because of a congenitally short or amputated cervix severe cicatricial changes left, for example by previous attempts at cerclage deep multiple cervical tears or cervicitis. Delivery is effected electively by Caesarean section.

The results of the treatment are good regardless of the method used. 60–85 per cent good results with improvement of foetal salvage of from 15–20 to 80 per cent have been reported (*Savarese* 1964 *Raphael* 1966)

Most authors recommend operation during pregnancy *i.e* some

type of cerclage, while cervical repair for an incompetent cervix in the non-pregnant patient should be reserved for cases in which a real anatomical defect is demonstrable, e.g. a cervical rupture. In such cases it is not sufficient to apply cerclage when pregnancy is established the cervical tear should be repaired during the non-pregnant stage, and during a subsequent pregnancy cerclage may be applied according to the usual principles.

It is stressed by most authors as an essential factor that the criteria of selection be strict and that surgical treatment should be done only in cases where the diagnosis is certain. The diagnosis cannot be made with certainty except in a pregnant patient who exhibits progressive painless cervical effacement and dilatation of the os exceeding 2–3 cm with protrusion of an intact amniotic sac. This applies regardless of the aetiology and of the outcome of other diagnostic tests carried out in the non-pregnant state (*Saverese* 1964).

The most favourable time for applying the cerclage is considered to be after the 14th week. At this juncture the cervix has not yet become softened and dilated and the first trimester is over. Thereby one avoids the risk of interfering with early abortions which are of a different genesis usually due to defective ova because of genetic damage or hormonal insufficiency.

Retrospective studies by Cushner (1963), *Goldstein* and *Wolff* (1964) and others have shown that the best results of cerclage are obtained among the group of patients in whom the diagnosis has been definitely established, in whom the cervix is <3 cm dilated, and who undergo an elective operation during the 14th–18th weeks.

If there is a greater degree of dilatation, where the amniotic sac protrudes, there may be a risk, due to amnionitis of infection causing foetal death and increased irritability of the uterus, leading to contractions.

A patient who is not seen until she is pregnant and shows a major degree of dilatation of the os possibly with a protruding amniotic sac should be put to bed with an elevated foot end and observed for 24–28 hours. If thereafter there is no tension of the membranes or progression of the abortion, closure of the cervix is justified.

In such cases cerclage may be difficult to apply and there may be a risk of accidental rupture of the amniotic sac.

Several methods of trachelorrhaphy have been described, said to be suited for closing such dilated cervices

Baden and *Baden* (1960) advocated the so-called bridge tracheloplasty suturing the external os, either longitudinally or transversely after excision of a strip of the epithelium on the ectocervix. *Heffner et al.* (1961) used the so-called Wurm procedure closing the os with 2 U sutures 1 transverse and 1 longitudinal.

However the simpler McDonald suture has proved applicable, also in major degrees of cervical dilatation, and it may be used with good results for elective as well as therapeutic procedures (*Quigley* and *Lynes* 1964)

After the cerclage operation the patient is kept in bed for 2–6 days and contractions if any are treated with morphine or spasmolytics. Prophylactic hormone therapy does not appear to be of any importance. The suture is left until the 36th–38th week unless delivery or abortion starts before that time. It is easy to remove by cutting, and as a rule the delivery occurs easily by the vaginal route. This applies especially after the McDonald procedure, while after the Shirodkar operation the rate of Caesarean section is quite high. This is partly explained by the fact that the Shirodkar operation is often of a more permanent nature aiming at preserving the cerclage for future pregnancies so that elective Caesarean section is carried out. Moreover the operation sometimes causes fibrous and cicatricial changes of the cervical tissue so that it may be difficult to remove the suture. Lastly because of the cicatricial tissue, the cervix cannot dilate and permit vaginal delivery so that a Caesarean section is required for this reason.

Raphael (1966) for instance had to deliver by Caesarean sections 18 out of 22 patients treated by the Shirodkar operation, *Landgren* and *Illeman* (1965) 7 out of 13 and *Strand* (1963) 8 out of 15

Among 6 patients treated by the Shirodkar procedure *Savarese* (1964) had 4 Caesarean sections and 2 vaginal deliveries

while among 9 patients treated by the McDonald procedure he had 2 sections and 7 vaginal deliveries.

Among *Quigley* and *Lynes* (1964) 24 deliveries at term after application of the McDonald suture 19 were vaginal and only 5 were by Caesarean section, including 4 on obstetrical indication unrelated to cervical incompetence. Among their series of 32 patients who underwent 38 operations 27 had been treated by the McDonald and 11 by the Shirodkar procedure.

Out of *McDonald's* (1957) 33 patients 31 had vaginal deliveries, while 2 had Caesarean sections for other obstetrical reasons.

Cerclage during pregnancy is contra-indicated if there has been drainage of amniotic fluid, if there is bleeding or contractions, if the os is dilated more than 3 cm, and if there is bulging of the amniotic sac with tension in the membranes.

Among Danielson's (1962) 14 patients treated by the Shirodkar operation 5 were treated in the acute state, and this failed to arrest the abortion in any case

Material and Methods

During the period December 1958 to December 1965 a total of 31 patients in the age range 22–40 years were treated for cervical incompetence

Table I lists the probable aetiology Special efforts were made to trace a traumatic genesis.

A history of trauma was found in 10 cases, while 6 patients had merely a history of an apparently normal delivery and in 15 cases there was no apparent cause

36 operations were performed, 34 cerclage procedures and 2 cervical repairs (Table II) The latter consisted in both cases of excision and suture of cervical tears in the non-pregnant state. One patient had 3 cerclage procedures + 1 cervical repair all during consecutive pregnancies.

In selecting the patients most emphasis was placed on an obstetrical history of 2 or more consecutive late abortions or premature deliveries of a course typical of incompetent cervix.

In such cases cerclage may be difficult to apply and there may be a risk of accidental rupture of the amniotic sac.

Several methods of trachelorrhaphy have been described, said to be suited for closing such dilated cervices

Baden and *Baden* (1960) advocated the so-called bridge tracheloplasty suturing the external os either longitudinally or transversely after excision of a strip of the epithelium on the ectocervix. *Heffner et al.* (1961) used the so-called Wurm procedure closing the os with 2 U sutures 1 transverse and 1 longitudinal.

However the simpler McDonald suture has proved applicable also in major degrees of cervical dilatation, and it may be used with good results for elective as well as therapeutic procedures (*Quigley* and *Lynes* 1964)

After the cerclage operation the patient is kept in bed for 2–6 days and contractions if any are treated with morphine or spasmolytics Prophylactic hormone therapy does not appear to be of any importance The suture is left until the 36th–38th week unless delivery or abortion starts before that time It is easy to remove by cutting, and as a rule the delivery occurs easily by the vaginal route This applies especially after the McDonald procedure while after the Shirodkar operation the rate of Caesarean section is quite high. This is partly explained by the fact that the Shirodkar operation is often of a more permanent nature aiming at preserving the cerclage for future pregnancies, so that elective Caesarean section is carried out. Moreover the operation sometimes causes fibrous and cicatricial changes of the cervical tissue, so that it may be difficult to remove the suture. Lastly because of the cicatricial tissue the cervix cannot dilate and permit vaginal delivery so that a Caesarean section is required for this reason.

Raphael (1966) for instance had to deliver by Caesarean sections 18 out of 22 patients treated by the Shirodkar operation, *Landgren* and *Illeman* (1965) 7 out of 13 and *Strand* (1963) 8 out of 15

Among 6 patients treated by the Shirodkar procedure *Savarese* (1964) had 4 Caesarean sections and 2 vaginal deliveries

while among 9 patients treated by the McDonald procedure he had 2 sections and 7 vaginal deliveries.

Among Quigley and Lynes (1964) 24 deliveries at term after application of the McDonald suture 19 were vaginal and only 5 were by Caesarean section, including 4 on obstetrical indication unrelated to cervical incompetence. Among their series of 32 patients who underwent 38 operations, 27 had been treated by the McDonald and 11 by the Shirodkar procedure.

Out of McDonald's (1957) 33 patients 31 had vaginal deliveries, while 2 had Caesarean sections for other obstetrical reasons.

Cerclage during pregnancy is contra-indicated if there has been drainage of amniotic fluid, if there is bleeding or contractions, if the os is dilated more than 5 cm, and if there is bulging of the amniotic sac with tension in the membranes

Among *Danielson*'s (1962) 14 patients treated by the Shirodkar operation 5 were treated in the acute state and this failed to arrest the abortion in any case.

Material and Methods

During the period December 1958 to December 1965 a total of 31 patients in the age range 22–40 years were treated for cervical incompetence

Table I lists the probable aetiology Special efforts were made to trace a traumatic genesis

A history of trauma was found in 10 cases, while 6 patients had merely a history of an apparently normal delivery and in 15 cases there was no apparent cause

36 operations were performed, 34 cerclage procedures and 2 cervical repairs (Table II) The latter consisted in both cases of excision and suture of cervical tears in the non-pregnant state. One patient had 3 cerclage procedures + 1 cervical repair all during consecutive pregnancies

In selecting the patients most emphasis was placed on an obstetrical history of 2 or more consecutive late abortions or premature deliveries of a course typical of incompetent cervix.

In such cases cerclage may be difficult to apply and there may be a risk of accidental rupture of the amniotic sac.

Several methods of trachelorrhaphy have been described, said to be suited for closing such dilated cervices

Baden and *Baden* (1960) advocated the so-called bridge tracheloplasty suturing the external os either longitudinally or transversely after excision of a strip of the epithelium on the ectocervix *Heffner et al.* (1961) used the so-called Wurm procedure closing the os with 2 U sutures 1 transverse and 1 longitudinal.

However the simpler McDonald suture has proved applicable, also in major degrees of cervical dilatation, and it may be used with good results for elective as well as therapeutic procedures (*Quigley* and *Lynes* 1964)

After the cerclage operation the patient is kept in bed for 2–6 days and contractions if any are treated with morphine or spasmolytics Prophylactic hormone therapy does not appear to be of any importance The suture is left until the 36th–38th week, unless delivery or abortion starts before that time. It is easy to remove by cutting, and as a rule the delivery occurs easily by the vaginal route This applies especially after the McDonald procedure while after the Shirodkar operation the rate of Caesarean section is quite high. This is partly explained by the fact that the Shirodkar operation is often of a more permanent nature aiming at preserving the cerclage for future pregnancies so that elective Caesarean section is carried out. Moreover the operation sometimes causes fibrous and cicatricial changes of the cervical tissue so that it may be difficult to remove the suture. Lastly because of the cicatricial tissue the cervix cannot dilate and permit vaginal delivery so that a Caesarean section is required for this reason.

Raphael (1966) for instance had to deliver by Caesarean sections 18 out of 22 patients treated by the Shirodkar operation, *Landgren* and *Illeman* (1965) 7 out of 13 and *Strand* (1963) 8 out of 15

Among 6 patients treated by the Shirodkar procedure *Savarese* (1964) had 4 Caesarean sections and 2 vaginal deliveries

Table III. 34 Cerclage Operations on 31 Patients during and before Pregnancy

Method	Therapeutic During Pregnancy	Prophylactic During Pregnancy	Befor Pregnancy	Total
Shirodkar	7	13	6	26
Mc Donald	6	2	0	8
	13	15	6	34

hysterographic demonstration of an increased width of the internal os.

The cerclage operations (Table II) were performed under general anaesthesia with the patient in the Trendelenburg position. 26 Shirodkar procedures were carried out. Vertical incisions in the epithelium of the anterior and posterior vaults were made. As a suture material we used a 5 mm Dacron tape applied subepithelially around the cervix, during traction by sponge forceps on the anterior and posterior cervical lips. The amniotic sac was pushed upwards, and the tape was applied circularly by a Dechamp needle on a level with the internal os and tied on the posterior aspect, where the ends were left jutting out 2 cm from the epithelial incision after the latter had been closed with catgut. Beforehand, the tape had been fastened anteriorly and posteriorly by two silk sutures.

8 cerclage operations were by the method of McDonald, using Dacron sutures or thick silk. Cerclage was done in the non-pregnant state in only 6 cases. This procedure is not advisable. A pregnancy should be awaited, and the cerclage should be deferred until the first trimester has passed.

Table III gives the distribution of the two methods as well as the distribution of therapeutic (acute) and prophylactic operations. It shows that 26 cerclage operations were by the method of Shirodkar and 8 by the method of McDonald. The distribution between acute and prophylactic procedures during pregnancy was 13/15.

In the non-pregnant state 6 cerclage operations were done.

The distribution of the 28 cerclage procedures during pregnancy is shown in Table IV. The majority were applied in the

Table I *Possible Aetiological Factors in 31 Patients with Cervical Incompetence*

Cervical rupture	2
Dilatation of cervix (dysmenorrhoea)	2
Dilatation of cervix (curettage)	1
Cervical pregnancy	1
Amputation of the cervix (precancerous)	1
Emmet s rupture	1
Electrocauterization of cervical canal	1
Forceful dilatation of cervix (atresia)	1
Obstetrical trauma and gynaec. surgery	10 (32.2 %)
Previous normal delivery	6 (19 4 %)
No apparent cause	15 (48.4 %)
	31

Table II. *Surgical Procedures on 31 Patients with Cervical Incompetence*
Age 22–40 years
Period Dec/58–Dec/65

	No of Patients	Cerclage	Total No. of Procedures	Trachelorrhaphy
	28	1 (28)	28	
	1	2 (2)	2	
	1	3 (3)	4	1
	1	1 (1)	2	1
Total	31	34	36	2

In 13 cases the diagnosis was based upon the condition of the cervix during the pregnancy in question, i.e softening, shortening, dilatation to more than 3 cm and/or visible bulging of the amniotic sac

In the 6 cases treated in the non-pregnant state the diagnosis had been confirmed during the course of previous pregnancy in 2 and in the remaining 4 by the obstetrical history plus the demonstration of an increased width of the internal os found by Hegar testing, as it admitted No 9–10 Hegar s dilators without resistance In one case the diagnosis was also supported by the

ADDENDUM TO TABLE OF CONTENTS OF VOL. XLVI, 1967

Table IV *28 Procedures Employed during Pregnancy*

	Total No of Procedures	Prophylactic	Therapeutic
First trimester	2	2	0
Second trimester	22	13	9
Third trimester	4	0	4
	28	15	13

Table V *Success Rate and Failures in 36 Procedures*

Total number of procedures	36
Successful outcome	27 (75%)
Failures	9 (25%)

Table VI *Course of Pregnancy in 31 Patients before and after Surgical Closure of Cervix*

	Number of Patients	Number of Pregnancies	Living Infants	Total		
				Third Trimester Termination	Second Trimester Termination	First Trimester Termination
Before treatment	31	85	1[illegible] (21%)	23 (27%)	45 (53%)	17 (20%)
After treatment	31	39	30 ([illegible]%)	33 ([illegible]4%)	5 (1[illegible]%)	1 (2[illegible]%)

second trimester and usually as prophylactic procedures. All 4 performed during the third trimester were done as acute operations.

The results of the 36 procedures assessed on the basis of the number that led to one or more successful pregnancies yielding live infants are presented in Table V. It will be seen that based upon this assessment 75 per cent were successful while 25 per cent failed. This is in keeping with the results of others. Shirodkar has reported 74 per cent success in [illegible]

Wiese J Acta obst et gynec scandinav 47 333, 1968

From the Department of Obstetrics and Gynaecology (*H Lefèvre M.D and J Weber M.D*) Copenhagen County Hospital in Glostrup Denmark

THE INDUCTION OF LABOUR USING SMALL TRANSBUCCAL DOSES OF SYNTOCINON

BY

J WIESE

Prior to 1943 Oxytocin was used for the induction of labour only by subcutaneous or intramuscular administration. *Page* (1943) introduced the intravenous method which was later developed by *Theobald* and his co-workers (1948) This method has a considerable number of advantages in comparison with those previously used, both with regard to effectiveness and safety The administration of Oxytocin can be discontinued immediately with the result that serious side-effects can be avoided, provided the patient is kept under close control.

One disadvantage with the method is that the patient must remain in bed, there is also the inconvenience of an intra enous infusion. An attempt has been made therefore to find other methods which cause fewer disadvantages for the patient without compromising the safety and effectiveness of the method.

Nasal application of Oxytocin has been used by among others *Hendricks et al.* (1960) *Borglin* (1962) *Clement et al* (1962) and *Cohn et al* (1962) but publications on the effectiveness are contradictory

In recent years there have been several reports of good results obtained using buccal administration of Oxytocin (*Dillon et al* 1960 *Rice et al* 1961 *Newman et al* 1963 *Elstein et al.* 1963 *Maxwell* 1964 *Blal* 1964 *Krzeslak* 1955 *and Harry* 1966) However publications have also reported rupture of the uterus (*Levy* 1964 *Nicholson* 1964 *O'Dwyer* 1964 and *Pinkerton* 1964) which has resulted in the method being condemned (*Bert*

M

N

O

S

T

W

Table III. Gestation in Weeks

	Buccal	Intravenous
38	3	5
38–39	7	17
40–41	45	43
42	45	35

Table IV. Success Rate *Analysed by State of Cervix and Parity*

Cervix	Total Number of Cases		Successful Cases	
	Buccal	Intravenous	Buccal	Intravenous
Ripe	81	78	70 (86 %)	72 (92 %)
Unripe	19	22	11 (58 %)	17 (77 %)
Parity				
Primigravida	50	46	33 (66 %)	37 (80 %)
Multipara	50	54	48 (96 %)	52 (96 %)

Material and Method

The series consists of 100 consecutive patients in whom labour was induced by intravenous infusion of Oxytocin and 100 patients in whom labour was induced by transbuccal application of a synthetic Oxytocin compound, Syntocinon (the drug has been kindly supplied by Sandoz A/S Basel Switzerland)

The indications for induction of labour can be seen in table I. Where several indications were present, only the main indication is given.

Table II shows the distribution according to age and parity

Table III shows the duration of the pregnancy and Table IV the state of the cervix. It can be seen that the two groups are comparable.

The syntocinon tablets were placed between the cheek and the upper gingiva, alternately on one side and then the other The patients were instructed not to suck or swallow the tablets.

One tablet (of 100 I.U.) was given each hour up to 5 times daily for 2 days or until the onset of labour If no effect was

Table I.

Indication for Induction	Buccal	Intravenous
Postmaturity	41	35
Toxaemia of pregnancy	6	17
Rupture of membranes without onset of labour	17	14
Pelvis contraction	13	10
Large foetus	6	10
Breech presentation	8	6
Placental insufficiency	3	2
Dead foetus	0	2
Unstable lie	2	0
Others	4	4
Total	100	100

Table II

Age	Primigravidae		Multiparae	
	Buccal	Intravenous	Buccal	Intravenous
15–19	6	6	1	
20–24	23	18	11	14
25–29	15	11	22	17
30–34	5	6	11	13
35–39	1	4	3	7
40–		1	2	3
Total	50	46	50	54

1964 and *Theobald* 1965) It has however been pointed out by *Pinkerton* (1964) that in four of the six cases of uterine rupture the use of Oxytocin by any route was contraindicated

Rather large doses of Oxytocin have been used in the studies published. *Pinkerton* (1964) and *Chalmers* (1964) advocate a reduction in the dosage

The object of the present study has been to examine the results following the induction of labour using small buccal doses of Oxytocin and comparing these with those obtained by intra venous infusion of Oxytocin

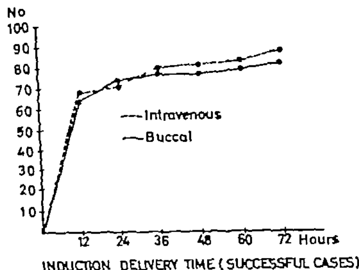


Fig. 1

cent delivered in the first day in both the buccal and intravenous series. The results were poorer in the buccal series in the following days.

In 4 and 6 cases respectively labour commenced, but owing to various factors not connected with the induction it was necessary to perform Caesarean section.

In 9 of the 15 cases regarded as failures labour was established by Oxytocin buccal tablets, but contractions were not optimal. Therefore although it was possible that additional buccal Oxytocin would have been successful, the induction was completed by the use of intravenous infusion.

There thus remain 6 patients who did not react to buccal Oxytocin. The indications for induction were in 5 cases postmature syndrome and in one spontaneous rupture of the membranes 5 women were primigravidae one was a tertigravida. The cervix was unripe in 3 patients. One patient was discharged after treatment for 2 days without an intravenous infusion being attempted. One did not react to intravenous infusion, and Caesarean section

Table V

Result of Induction	Buccal	Intravenous
Success 1 day's administration	69	69
Success 2 day s administration	9	12
Success 3 day s administration	3	4
Success 4 day s administration	0	4
Failures	15	5
Labour established but delivered by Caesarean section	4	6
Total	100	100

Table VI *Average Dosage of Syntocinon (I U)*

	Buccal	Intravenous
Successful cases	496	8
Unsuccessful cases	805	10
All induction	556	8

obtained, one day s pause was allowed and thereafter a new attempt was made on 2 consecutive days After this the induction was either abandoned or an attempt made to induce labour by the use of intravenous infusion of Oxytocin It was normally possible to cease the administration of the tablets when regular uterine contractions were established.

The membranes were ruptured artificially in 51 per cent of the cases in the intravenous and 56 per cent in the buccal series but not unless the patient was undoubtedly in labour

The blood pressure uterine contractions and foetal heart rate were carefully controlled in both the patients subjected to Oxytocin intravenously and those given buccal tablets

Results

It can be seen from Table V that as judged by achievement of vaginal delivery there were 81 per cent successful results in the buccal and 89 per cent in the intravenous series. Sixty nine per

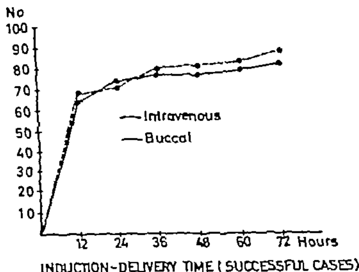


Fig. 1

cent delivered in the first day in both the buccal and intravenous series. The results were poorer in the buccal series in the following days.

In 4 and 6 cases respectively labour commenced, but owing to various factors not connected with the induction it was necessary to perform Caesarean section.

In 9 of the 15 cases regarded as failures labour was established by Oxytocin buccal tablets, but contractions *were not* optimal. Therefore, although it was possible that additional buccal Oxytocin would have been successful the induction was completed by the use of intravenous infusion.

There thus remain 6 patients who did not react to buccal Oxytocin. The indications for induction were in 5 cases postmature syndrome and in one spontaneous rupture of the membranes 5 women were primigravidae one was a tertigravida. The cervix was unripe in 3 patients. One patient was discharged after treatment for 2 days, without an intravenous infusion being attempted. One did not react to intravenous infusion, and Caesarean section

Table V

Result of Induction	Buccal	Intravenous
Success 1 day s administration	69	69
Success 2 day s administration	9	12
Success 3 day s administration	3	4
Success 4 day s administration	0	4
Failures	15	5
Labour established but delivered by Caesarean section	4	6
Total	100	100

Table VI. *Average Dosage of Syntocinon (I U)*

	Buccal	Intravenous
Successful cases	496	8
Unsuccessful cases	805	10
All induction	556	8

obtained, one day s pause was allowed and thereafter a new at tempt was made on 2 consecutive days. After this the induction was either abandoned or an attempt made to induce labour by the use of intravenous infusion of Oxytocin. It was normally pos sible to cease the administration of the tablets when regular uterine contractions were established.

The membranes were ruptured artificially in 51 per cent of the cases in the intravenous and 56 per cent in the buccal series but not unless the patient was undoubtedly in labour

The blood pressure uterine contractions and foetal heart rate were carefully controlled in both the patients subjected to Oxy tocin intravenously and those given buccal tablets.

Results

It can be seen from Table V that as judged by achievement of vaginal delivery there were 81 per cent successful results in the buccal and 89 per cent in the intravenous series Sixty nine per

In 13 of the 17 cases of foetal distress in the intravenous series the distress was a transient feature and the Apgar score lay between 7 and 10 In one case the foetal heart rate was affected by a prolonged contraction. In an additional two cases the foetal heart rate did not return to normal, and Caesarean section was performed. In the two remaining cases there was no apparent connection between the foetal distress and intravenous Oxytocin. All of the 17 patients were delivered vaginally

There was no case of uterine rupture. In one case in the buccal and two in the intravenous series there was increased tonus of the uterus which quickly disappeared after removal of the tablet and washing of the mouth or cessation of the infusion.

The blood loss during birth was above 500 ml in two cases in the buccal and four in the intravenous series.

Discussion

The results (81 per cent successful in the buccal series) are on a par with the other reported results of other materials *Dillon et al.* (1960) have thus 70 per cent positive *Rice et al* (1961) 76 per cent *Elstein et al* (1963) 78 per cent *Maxwell* (1964) 89 per cent, *Blair* (1964) 65 per cent and *Harry* (1966) 87 per cent. Our average consumption of Oxytocin (556 I.U) is, however considerably lower than that of the above mentioned investigators which were respectively 1157 2100 4400 2094 3000 and 6380 I U Oxytocin.

Our results correspond almost exactly to those of *Chalmers et al.* (1966) whose investigation was published after our study had been commenced. These authors have successively reduced the dose from 4400 to 535 I U without a reduction in the effectiveness There were 80 per cent positive results with the last mentioned dose

In the intravenous series there were 89 per cent positive results against 81 per cent in the buccal series. *Maxwell's* figures from 1964 were 90 per cent and 89 per cent. In the buccal series labour was established in an additional 9 per cent patients, but, as the contractions became weaker intravenous infusion was given instead of repeating the buccal administration.

Table VII. *Time from Induction to Onset of Labour*

Hours	0–1	1–2	2–3	3–4	4–6	6–
Buccal	13	14	12	16	14	25
Intravenous	27	19	12	5	8	24

Table VIII *Incidence of Foetal Distress*

	Buccal	*Intravenous*
Foetal heart irregularity	8	17
Apgar scores lower than 7	4	7

was performed. In the remaining 4 patients the birth was com pleted by means of intravenous infusion of Oxytocin.

From Table IV it can be seen that it is particularly in cases of unripe cervix and in primigravidae that the results of intravenous *administration* are better

As can be seen from Table VI the average dose of Oxytocin in the buccal series was 556 I U The maximum dose was 1500 the minimum dose 100 I.U

Table VII and Fig 1 show the time intervals from the com mencement of the induction to the onset of labour and to deliv- ery The onset of labour occurred within 6 hours in 69 patients in the buccal and in 71 in the intravenous series and the birth *was completed within 12 hours in 64 and 68 patients respectively*

No *maternal deaths occurred*. Two still born children were both dead prior to the induction.

The number of cases in which the foetus was adversely af fected is shown in Table VIII In 6 of the 8 cases in the buccal series the foetal heart rate was only affected for a short period and the Apgar score lay between 7 and 10 One case was pre- sumed to be caused by a brief increase in tonus of the uterus In the remaining case it was considered that the foetal distress had no connection with the buccal Oxytocin. Vaginal delivery oc- curred in all 8 cases

Small doses of Oxytocin given buccally have thus been seen to be effective and presumably the risk of uterine hypertonia is less with the smaller the Oxytocin dosage used in this series.

REFERENCES

Bent F A. Brit. med. J 2 842, 1964
Blair R G Lancet, 1 637 1964
Borglin N E Acta obst. et gynec. scandinav 41 238 1962
Chalmers J A and NG J L, Brit. med. J 2 1070 1964
Chalmers J A. and *Moorhouse H M.* J Obstet. Gynaec. Brit. Cwlth 73 59 1966
Clemetson J E. Hereell V D and *Mc Cabe R.* Am. J Obst. & Gyn., 83 778, 1962
Cohen J *Dewerts J* and Barnhill M. S Am. J Obst. & Gyn. 83 774 1962
Dillon T F *Douglas R. G du Vigneaud V* and Barber M. L. Obst. Gynec 15 587 1960
O'Dwyer E M Brit. med. J 2 1133 1964
Elstein M and *Fayting Wright H* J Obstet. Gynaec. Brit Cwlth., 70 6 1963
Harry J M Ugeskrift for Laeger 12 345 1966
Hendricks H E and Gebel R. A. Am. J Obst. & Gyn 79 780 1960
Krzowak, S Irish med Association, 56 113 1965
Lewry P M Brit. med. J 2 689 1964
Maxwell A. W J Obstet Gynaec. Brit. Cwlth. 71 37 1964
Newmann J W and *Hon E H* Obstet. and Gynec., 21 3 1963
Nicholson H O Brit. med J 2 876 1954
Page E. W Proc Soc exp Biol (N Y) 52 195 1943
Pinkerton J H M Brit. med. J 2 1393 1964
Rice R D and Benson R. C Obstet. Gynec. 17 297 1961
Theobald, G W *Graham A Campbell, J Gange P D* and *Driscoll* W J Brit med J 2 123, 1948
Theobald, G W Brit med. J 1 190 1965

Received on May 30 1967

In the intravenous series there were two cases and in the buccal series one case of uterine hypertonia which quickly dis appeared *Neumann et al* (1963) also found that the activity of the uterus decreased just as quickly after the cessation of buccal as after intravenous Oxytocin.

Foetal distress was more frequent in the intravenous than in the buccal series

The buccal method is more pleasant for the patient than the intravenous method, and more convenient for the staff But it must be pointed out that the indications and contraindications must be complied with and that induction must take place at a hospital where the patient can be subjected to close control and where facilities are available for treating any possible complica tions

Providing the above is adhered to we think that Oxytocin administered buccally in small doses as suggested is a suitable and safe method for the induction of labour

SUMMARY

The induction of labour using small doses of Oxytocin buccally was attempted, and the results of 100 consecutive cases were compared with 100 consecutive cases in which the labour was induced by the use of intravenous infusion of Oxytocin

The induction was successful in 81 per cent in the buccal and 89 per cent in the intravenous series. An additional 9 per cent could presumably have been carried through by means of buccal Oxytocin.

There were no cases of maternal or foetal mortality ascribed to the procedure

Foetal distress was more frequent in the intravenous than in the buccal series

There was one case of uterine hypertonia in the buccal and two in the intravenous series

Buccal administration is more convenient for the patient and more easy to administer

The average dosage of Oxytocin in the buccal series was 556 I U

without clots or impressions. The umbilical cord was almost centrally inserted and of normal length. The membranes were quite dark red due to clots covering the foetal surface of the placenta between the amnion and chorion and extending throughout the whole space between these two membranes. On the foetal surface of the placenta no damage could be demonstrated to the vessels. The autopsy of the child revealed nothing except for anaemia and discrepancy between weight and length (2400 g 53 cm). The woman herself was in a normal condition all the time. She was discharged five days later with a haemoglobin of 15 g/100 cc.

Discussion

Four cases similar to ours have been reported (*Frech* 1949 *Leff* 1931 *Matthews* 1961 *Renneberg*, 1924). In the case of *Frech* a living child was born thanks to the fact that conditions at the time of the haemorrhage were so favourable that a delivery by forceps could be performed at once. In the three other cases stillborn babies were delivered.

Renneberg considered that the bleeding was into the amniotic cavity itself because the haemorrhage occurred at the time of rupture of the membranes. But the three other writers describe clotting between the membranes as in the present case, which indicates that the blood does not get into the amniotic cavity. I find that our case gives the best proof of this for in this case the membranes were ruptured one by one firstly revealing a large haematoma, secondly normal amniotic fluid.

All *four* writers find a discontinuity of the wall of a placental vein in relation to a small varicosity on the foetal surface. In two cases the hole could not be seen until the umbilical vein was injected. In our case the placenta unfortunately was put into formalin shortly after the delivery. By macroscopical examination the site of the haemorrhage could not be seen and there were no varices visible. No attempt was made to demonstrate a vascular defect by the method of injection into the umbilical vein.

Wentworth (1965) has examined 860 consecutive placentae and in 1.95 per cent found either aneurysms (7) or varices (10) of the chorionic vessels. None of these dilatations ruptured after having been submitted to a pressure of 500 mm mercury. This explains that these not extremely uncommon abnormalities are

Gad, Claus, Acta obst. et gynec. scandinav 47 342, 1968
From the Department of Surgery (B Helberg, M.D and A. Bindslev M.D)
Central Hospital Holbaek Denmark

A CASE OF HAEMORRHAGE IN LABOUR FROM A PLACENTAL VESSEL CAUSING FOETAL DEATH

BY

CLAUS GAD

It is very uncommon to see haemorrhage of foetal origin during pregnancy and labour This case report concerns a haemorrhage from a placental vessel with the blood emerging between the membranes

Case

On the 13th April 1967 at 4 15 p.m a married woman aged 23 was admitted to hospital at term in her first pregnancy Three weeks previously she had very slight vaginal bleeding On the day before admission she had felt foetal movements and the midwife had heard normal hearts beats. The blood pressure had been 145/90 and slight periorbital oedema was noticed there was no oedema of the legs

At about 3 45 p.m. she called the midwife having felt some sensations of the abdomen. No foetal heart sounds could be detected. On vaginal exploration a tense membrane was felt, which happened to burst Immediately there was a gush of dark red or brown liquid estimated at 500-700 cc from the vagina. The woman was admitted at once

When admitted to hospital she was in very god condition with a normal pulse rate and blood pressure Haemoglobin was 13 g per 100 cc No foetal heart sounds were heard The uterine fundus nearly reached the xiphoid and the foetal head was well engaged. There was a small amount of blood in the vagina. On vaginal exploration an os of 4-5 cm was found the membranes seemed to be intact They were ruptured in order to induce labour The amniotic fluid was clear No placental tissue could be demonstrated on exploration. Labour commenced quickly At 6 p m. the second stage began and at 6 45 p.m a stillborn boy was born in a normal occipital presentation There was no heart action or respiration and resuscitation was futile Five minutes later the placenta was delivered The maternal surface was normal

without clots or impressions. The umbilical cord was almost centrally inserted and of normal length. The membranes were quite dark red due to clots covering the foetal surface of the placenta between the amnion and chorion and extending throughout the whole space between these two membranes. On the foetal surface of the placenta no damage could be demonstrated to the vessels. The autopsy of the child revealed nothing except for anaemia and discrepancy between weight and length (2400 g 53 cm) The woman herself was in normal condition all the time. She was discharged five days later with haemoglobin of 15 g/100 cc.

Discussion

Four cases similar to ours have been reported (*Frech* 1949 *Leff* 1931 *Matthews* 1961 *Ranneberg*, 1924) In the case of Frech a living child was born thanks to the fact that conditions at the time of the haemorrhage were so favourable that a delivery by forceps could be performed at once. In the three other cases stillborn babies were delivered.

Ranneberg considered that the bleeding was into the amniotic cavity itself because the haemorrhage occurred at the time of rupture of the membranes. But the three other writers describe clotting between the membranes as in the present case which indicates that the blood does not get into the amniotic cavity I find that our case gives the best proof of this for in this case the membranes were ruptured one by one, firstly revealing a large haematoma, secondly normal amniotic fluid.

All four writers find a discontinuity of the wall of a placental vein in relation to a small varicosity on the foetal surface In two cases the hole could not be seen until the umbilical vein was injected. In our case the placenta unfortunately was put into formalin shortly after the delivery By macroscopical examination the site of the haemorrhage could not be seen and there were no varices visible. No attempt was made to demonstrate a vascular defect by the method of injection into the umbilical vein.

Wentworth (1965) has examined 860 consecutive placentae and in 1.95 per cent found either aneurysms (7) or varices (10) of the chorionic vessels None of *these dilatations* ruptured after having been submitted to a pressure of 500 mm mercury This explains that these not extremely uncommon abnormalities are

Gad, Claus, Acta obst. et gynec. scandinav. 47 342, 1968

From the Department of Surgery (B. Helberg, M.D. and A. Bindslev M.D.)
Central Hospital Holbæk Denmark

A CASE OF HAEMORRHAGE IN LABOUR FROM A PLACENTAL VESSEL CAUSING FOETAL DEATH

BY

CLAUS GAD

It is very uncommon to see haemorrhage of foetal origin during pregnancy and labour This case report concerns a haemorrhage from a placental vessel with the blood emerging between the membranes

Case

On the 13th April 1967 at 4 15 p.m. a married woman aged 23 was admitted to hospital at term in her first pregnancy Three weeks previously she had very slight vaginal bleeding On the day before admission she had felt foetal movements and the midwife had heard normal hearts beats The blood pressure had been 145/90 and slight periorbital oedema was noticed there was no oedema of the legs

At about 3 45 p.m. she called the midwife having felt some sensations of the abdomen. No foetal heart sounds could be detected On vaginal exploration a tense membrane was felt, which happened to burst. Immediately there was a gush of dark ed or brown liquid estimated at 500–700 cc from the vagina The woman was admitted at once.

When admitted to hospital she was in very god condition with a normal pulse rate and blood pressure Haemoglobin was 13 g per 100 cc No foetal heart sounds were heard. The uterine fundus nearly reached the xiphoid and the foetal head was well engaged. There was a small amount of blood in the vagina. On vaginal exploration an os of 4–5 cm was found, the membranes seemed to be intact. They were ruptured in order to induce labour The amniotic fluid was clear No placental tissue could be demonstrated on exploration. Labour commenced quickly At 6 p.m. the second stage began and at 6 45 p.m a stillborn boy was born in a normal occipital presentation There was no heart action or respiration and resuscitation was futile Five minutes later the placenta was delivered. The maternal surface was normal

Odeblad, Erik and Rosenberg, Birgitta, Acta obst. et gynec. scandinav. 47 345, 1968

From the Department of Medical Physics (Prof E. Odeblad) University of Umeå Sweden

A LOW VISCOSITY COMPONENT IN HUMAN UTERINE ENDOCERVICAL CONTENTS

BY

ERIK ODEBLAD AND BIRGITTA ROSENBERG

We are studying a low viscosity component present in the secretion of human uterine cervix. During a period of about a year the low-viscosity component was searched for in seventeen healthy women and was observed in all of them. It can easily be distinguished by its physical properties from the commonly obtained cervical secretion. It is a yellow fluid of low viscosity and can easily be collected from the external os in thin glass tubes utilizing capillary force. The usual cervical secretion cannot be collected in this way because of its high viscosity. It was also found that the low viscosity material was available in especially large quantities after application of certain cervical or other stimuli while the high-viscosity secretion was probably not augmented by these stimuli.

Pure low viscosity secretion has been collected as follows.

1) The portio was made accessible with a self holding speculum and inspected with the colposcope.
2) All vaginal contents were removed from the portio and external os.
3) The high-viscosity cervical secretion was sampled, using aspiration in 2 3 mm I. D wide glass tubes.
4) A standardized mechanical stimulation was carried out by applying cotton swabs to the cervical canal for one minute.
5) The low-viscosity secretion was sampled with glass capillaries (0.2–0.5 mm I. D) under colposcopic control (Fig. 1) for several consecutive periods each of approximately one minute

very unlikely to cause haemorrhage Wentworth also found four cases of subchorionic extravasation only in one case covering more than half of the surface In two cases a pin hole rupture was found in a chorionic vein in the remaining two cases no origin of the haemorrhage could be traced. No varix or aneurysm was present and it was considered that the bleeding, which in all cases was recent occurred in the third stage. No clinical histories are given.

Potter (1962) has mentioned that haemorrhage from vessels of the foetal surface of placenta will emerge between the mem branes

Another cause of foetal bleeding is rupture of a vasa praevia. This condition is combined with a velamentous insertion of the cord. Although it is not very common more than 100 cases have been reported the latest three by *Naftolin et al.* (1965)

SUMMARY

A rare case of haemorrhage from a placental vessel is reported. In the literature only four previous cases have been published, with which the present one is compared

REFERENCES

Frech H C Am. J Obst. & Gynec. 57 1024 1949
Leff M Am. J Obst. & Gynec. 22 117 1931
Matthews A. E. B J Obst. & Gynec. 68 834 1961
Naftolin F and *Mishell D R.* Obst. & Gynec. 26 561 1965
Potter E. L. Pathology of the Fetus and Infant 2nd ed p. 24 1962
Ranneberg, E Zbl. Gynäk 48 30 1924
Wentworth P J Anat 99 273 1965

Received on Aug 29 1967



Fig. 2. Sampling diagram of low-viscosity secretion. The ordinate shows the recovered in mg/sec. Abscissa indicates time in minutes. S denotes cervical stimulation. The diagram shows marked increase in production rate following cervical stimulation.

C) Wet and dried preparations have been studied microscopically. The presence of cells and other morphological constituents has been noted. Only a few epithelial cells and red blood cells have occasionally been seen. A variety of crystal formations probably NaCl, were always present in dried secretion.

D) Protein reactions were performed and were strongly positive.

E) Paper electrophoresis was performed. For comparison, high-viscosity cervical secretion, albumin gamma globulin and blood serum were also tested. The investigations have shown that albumin was regularly present and that alpha, beta and gamma globulin components were usually present (Fig. 3).

F) Dried low-viscosity secretion was investigated with EPR and compared with several purified proteins and did not show the presence of specific absorption lines.

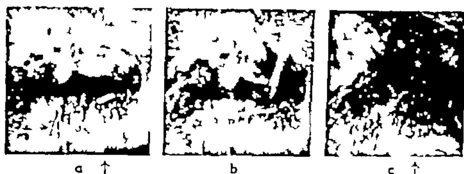


Fig. 1 Photo A shows the external os with light reflection (at arrow) on the surface of low-viscosity secretion. Photo B shows a glass capillary introduced in the secretion. Photo C shows the external os after the fluid has been removed with the glass capillary Light reflection at arrow is absent.

We have applied the standardized mechanical stimulation with cotton swabs in the cervical canal and followed the production of low viscosity material during regular menstrual cycles The available quantities of low-viscosity secretion seem to vary in the different phases The rate of formation is lower premenstrually than during the other phases

When performing the clinical investigations the exact times of sampling were noted and the amounts were determined by weighing The data was recorded in diagrams actually as shown in Figure 2 A total of about 600 individual samples of low viscosity secretion have been collected and recorded in this way

We have also data indicating that the production rate may be augmented or diminished by different stimuli Removal of conventional cervical samples dislocation of the portio breast stimulation and other factors have been studied.

In order to analyze the components present in the low viscosity secretion a number of investigations have been performed Some of them are the following

A) *Gross composition.* The total content of solid material was found to be about 5 per cent while the remainder is made up of water

B) Sodium ions have been identified by flame spectroscopy and chloride ions with silver chloride precipitation



Fig. 2. Sampling diagram of low-viscosity secretion. The ordinate shows the recovered in mg/sec. Abscissa indicates time in minutes. S denotes cervical stimulation. The diagram shows marked increase in production rate following cervical stimulation.

C) Wet and dried preparations have been studied microscopically. The presence of cells and other morphological constituents has been noted. Only a few epithelial cells and red blood cells have occasionally been seen. A variety of crystal formations probably NaCl were always present in dried secretion.

D) Protein reactions were performed and were strongly positive.

E) Paper electrophoresis was performed. For comparison, high-viscosity cervical secretion, albumin, gamma globulin and blood serum were also tested. The investigations have shown that albumin was regularly present and that alpha beta and gamma globulin components were usually present (Fig. 3).

F) Dried low viscosity secretion was investigated with EPR and compared with several purified proteins and did not show the presence of specific absorption lines.



Fig. 1 Photo A shows the external os with light reflection (at arrow) on the surface of low-viscosity secretion. Photo B shows a glass capillary introduced in the secretion. Photo C shows the external os after the fluid has been removed with the glass capillary Light reflection at arrow is absent.

We have applied the standardized mechanical stimulation with cotton swabs in the cervical canal and followed the production of low viscosity material during regular menstrual cycles The available quantities of low viscosity secretion seem to vary in the different phases The rate of formation is lower premenstrually than during the other phases

When performing the clinical investigations the exact times of sampling were noted and the amounts were determined by weighing The data was recorded in diagrams actually as shown in Figure 2. A total of about 600 individual samples of low viscosity secretion have been collected and recorded in this way

We have also data indicating that the production rate may be augmented or diminished by different stimuli Removal of conventional cervical samples dislocation of the portio breast stimulation and other factors have been studied

In order to analyze the components present in the low viscosity secretion a number of investigations have been performed Some of them are the following

A) Gross composition The total content of solid material was found to be about 5 per cent while the remainder is made up of water
B) Sodium ions have been identified by flame spectroscopy and chloride ions with silver chloride precipitation

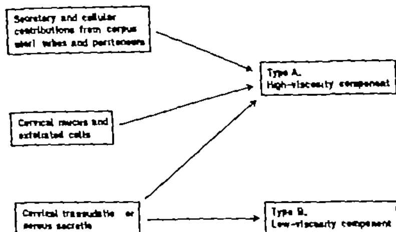


Fig 4 A schematic diagram showing the origin and composition of cervical secretions.

A number of different aspects of cervical secretion type B are presently under study at our department.

Acknowledgment

We want to express our thanks to Hoffman-La Roche Co Basel, Switzerland for support in this investigation.

REFERENCES

Benes A Radovi med. fak u Zagreb., 198, 1958

Newhouse O W and *Moghissi K. S* Fert. and Ster 13 550 1962

Received on Aug 2, 1957

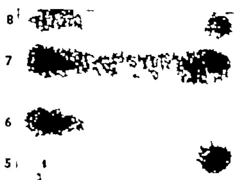


Fig 3 Electrophoresis pattern of albumin (5) gamma globulin (6) low viscosity secretion (7) and a mixture of albumin and gamma globulin (8). The electrophoresis indicates the presence of albumin alpha beta, and gamma globulins in the low viscosity material.

The composition of the low viscosity secretion suggests that it is formed by a process of transudation or serous glandular secretion. It must be pointed out that the high viscosity secretion contains low viscosity material in small and varying amounts together with cervical mucoid, endometrial secretion and other contributions (Fig 4) It has previously been shown (*Benas* 1958 and *Neuhauss* and *Moghissi* 1962) that serum protein constituents are present in regular cervical samples. We have been able to collect the protein constituents in pure form at their moment of appearance in the external os In our laboratory we have adopted the notation "Secretion type A for the mixed material while the pure low viscosity material is called Secretion type B

Spermigration tests indicate that spermigration occurs in the low viscosity secretion and in ordinary cervical samples as well as in mixtures of the two types It is not impossible that secretion type B may have physiological or pathological significance for the motility of spermatozoa Antisperm antibodies may be present in the gamma globulin fraction of the secretion type B *In vitro* there occurs a rapid mixing of types A and B which has also been found *in vivo*

All pertinent data suggest that secretion type B does not originate from higher regions of the genital tract but from the endocervix.

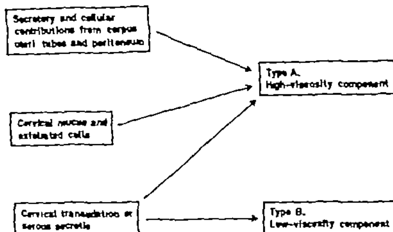


Fig. 4. A schematic diagram showing the origin and composition of cervical secretions.

A number of different aspects of cervical secretion type B are presently under study at our department.

Acknowledgment

We want to express our thanks to Hoffman-La Roche Co Basel, Switzerland, for support in this investigation.

REFERENCES

Bruns, A Radovi med. fak. Zagreb., 198, 1958
Neuhaus O W and Moghissi K. S Fert. and Ster 13 550 1962

Received on Aug 2, 1957

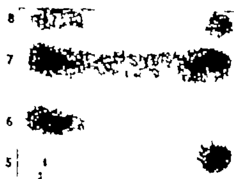


Fig 3 Electrophoresis pattern of albumin (5) gamma globulin (6) low viscosity secretion (7) and a mixture of albumin and gamma globulin (8) The electrophoresis indicates the presence of albumin alpha beta and gamma globulins in the low-viscosity material.

The composition of the low viscosity secretion suggests that it is formed by a process of transudation or serous glandular secretion. It must be pointed out that the high viscosity secretion contains low viscosity material in small and varying amounts together with cervical mucoid, endometrial secretion and other contributions (Fig 4) It has previously been shown (*Benas* 1958 and *Neuhauss* and *Moghissi* 1962) that serum protein constituents are present in regular cervical samples We have been able to collect the protein constituents in pure form at their moment of appearance in the external os In our laboratory we have adopted the notation "Secretion type A for the mixed material while the pure low viscosity material is called Secretion type B

Spermigration tests indicate that spermigration occurs in the low viscosity secretion and in ordinary cervical samples as well as in mixtures of the two types It is not impossible that secretion type B may have physiological or pathological significance for the motility of spermatozoa Antisperm antibodies may be present in the gamma globulin fraction of the secretion type B *In vitro* there occurs a rapid mixing of types A and B which has also been found *in vivo*

All pertinent data suggest that secretion type B does not originate from higher regions of the genital tract but from the endocervix.

We are able to review 85 patients with major ovarian endometriosis who had been laparoscoped immediately or some time before laparotomy

The clinical diagnoses i.e. the tentative diagnoses before laparoscopy were the following (Table I)

Table I. *Clinical Diagnosis Before Laparoscopy*

I. *Endometriosis*	39
II. Ovarian tumour	
] ovarium tumour	8
b) fibroid or/and ovarian tumour	13
III *Acute abdomen*	
a) acute salpingitis	8
b) acute abdominal pain	2
) ectopic pregnancy	4
IV Chronic abdominal pain	3
V Sterility	8
	85

By clinical diagnosis is here understood the tentative prelaparoscopic diagnosis founded in the usual way on the case history and the various symptoms and signs present.

The final decision regarding whether a laparoscopy should be performed or not, was generally taken after examining the patient under anaesthesia.

The clinical prelaparoscopic diagnosis was almost always specially noted on the case-sheet beforehand. In some cases from earlier years only before this rule was strictly adopted, we were also without too much difficulty able to decide on the clinical diagnosis which led to laparoscopic examination.

We classified our cases according to the clinical prelaparoscopic diagnosis. This was done because it became obvious during the investigation that laparoscopists decisions were at times biased towards agreement with the clinical prelaparoscopic diagnoses.

The laparoscopic examinations were performed in the usual way under general anaesthesia, always using carbon dioxide to

Samuelsson, Stig and Sjövall, Alf Acta obst. et gynec. scandinav 47 350, 1968

From the Department of Obstetrics and Gynaecology (Prof Alf Sjövall) University Hospital Lund Sweden

ON THE DIAGNOSTIC VALUE OF LAPAROSCOPY IN OVARIAN ENDOMETRIOSIS

BY

STIG SAMUELSSON AND ALF SJÖVALL

Since 1948 laparoscopy has been used at the Department of Obstetrics and Gynaecology in Lund with increasing frequency Since 1961 it has become an almost daily routine procedure performed by all sufficiently experienced members of the staff The number of laparoscopic examinations 1948–1965 amounts to 1834 of which 1348 were performed during the years 1961–1965

Although laparoscopy—like other endoscopic methods—can give *almost instantly a firm and conclusive diagnosis* full use of the diagnostic possibilities of laparoscopy may depend upon thoroughness in the inspection and manipulation of the intra abdominal structures

Our experience suggested that, in the laparoscopic diagnosis of ovarian endometriosis the laparoscopist often did not arrive at the correct conclusion, overlooking certain characteristics of the lesion drawing too swift conclusions from what was immediately seen and stopping the *inspection and manipulation too soon.*

To assess the diagnostic value of laparoscopy in ovarian endometriosis we have picked out those cases where ovarian endometriosis was proven beyond doubt by a subsequent laparotomy during the years 1948–1965 Included in the investigation are cases with comparatively extensive involvement of the substance of the ovary *i e* chocolate cysts Cases presenting only small patches of endometriosis on the ovarian surface and no further involvement were excluded. *They do not present a* diagnostic problem.

We are able to review 85 patients with major ovarian endometriosis who had been laparoscoped immediately or some time before laparotomy

The clinical diagnoses i.e. the tentative diagnoses before laparoscopy were the following (Table I)

Table I. *Clinical Diagnosis Before Laparoscopy*

I. Endometriosis	39
II. Ovarian tumour	
a) ovarian tumour	8
b) fibroid or/and ovarian tumour	13
III. *Acute abdomen*	
a) acute salpingitis	8
b) acute abdominal pain	2
c) ectopic pregnancy	4
IV. Chronic abdominal pain	3
V. Sterility	8
	85

By clinical diagnosis is here understood the tentative prelaparoscopic diagnosis founded in the usual way on the case history and the various symptoms and signs present.

The final decision regarding whether a laparoscopy should be performed or not, was generally taken after examining the patient under anaesthesia.

The clinical prelaparoscopic diagnosis was almost always specially noted on the case-sheet beforehand. In some cases from earlier years only before this rule was strictly adopted, we were also without too much difficulty able to decide on the clinical diagnosis which led to laparoscopic examination.

We classified our cases according to the clinical prelaparoscopic diagnosis. This was done because it became obvious during the investigation that laparoscopists decisions were at times biased towards agreement with the clinical prelaparoscopic diagnoses.

The laparoscopic examinations were performed in the usual way under general anaesthesia, always using carbon dioxide to

Samuelsson, Stig and Sjövall, Alf Acta obst. et gynec. scandinav 47 390, 1968

From the Department of Obstetrics and Gynaecology (Prof Alf Sjövall)
University Hospital Lund Sweden

ON THE DIAGNOSTIC VALUE OF LAPAROSCOPY IN OVARIAN ENDOMETRIOSIS

BY

STIG SAMUELSSON AND ALF SJÖVALL

Since 1948 laparoscopy has been used at the Department of Obstetrics and Gynaecology in Lund with increasing frequency Since 1961 it has become an almost daily routine procedure performed by all sufficiently experienced members of the staff The number of laparoscopic examinations 1948–1965 amounts to 1834 of which 1348 were performed during the years 1961–1965

Although laparoscopy—like other endoscopic methods—can give almost instantly a firm and conclusive diagnosis full use of the diagnostic possibilities of laparoscopy may depend upon thoroughness in the inspection and manipulation of the intra abdominal structures

Our experience suggested that, in the laparoscopic diagnosis of ovarian endometriosis the laparoscopist often did not arrive at the correct conclusion, overlooking certain characteristics of the lesion drawing too swift conclusions from what was immediately seen and stopping the inspection and manipulation too soon

To assess the diagnostic value of laparoscopy in ovarian endometriosis we have picked out those cases where ovarian endometriosis was proven beyond doubt by a subsequent laparotomy during the years 1948–1965 Included in the investigation are cases with comparatively extensive involvement of the substance of the ovary *i e* chocolate cysts Cases presenting only small patches of endometriosis on the ovarian surface and no further involvement were excluded. They do not present a diagnostic problem

We are able to review 85 patients with major ovarian endometriosis who had been laparoscoped immediately or some time before laparotomy

The clinical diagnoses, i.e. the tentative diagnoses before laparoscopy were the following (Table I)

Table I *Clinical Diagnosis Before Laparoscopy*

I. Endometriosis	38
II. Ovarian tumour	
) ovarian tumour	8
b) fibroid or/and ovarian tumour	13
III *Acute abdomen*	
] acute salpingitis	8
b) acute abdominal pain	2
] ectopic pregnancy	4
IV Chronic abdominal pain	3
V Sterility	8
	85

By clinical diagnosis is here understood the tentative pre laparoscopic diagnosis founded in the usual way on the case history and the various symptoms and signs present.

The final decision regarding whether a laparoscopy should be performed or not, was generally taken after examining the patient under anaesthesia.

The clinical prelaparoscopic diagnosis was almost always specially noted on the case-sheet beforehand. In some cases from earlier years only before this rule was strictly adopted, we were also without too much difficulty able to decide on the clinical diagnosis which led to laparoscopic examination.

We classified our cases according to the clinical prelaparoscopic diagnosis This was done because it became obvious during the investigation that laparoscopists decisions were at times biased towards agreement with the clinical prelaparoscopic diagnoses.

The laparoscopic examinations were performed in the usual way under general anaesthesia, always using carbon dioxide to

distend the abdominal cavity A volsellum forceps or a cannula for hydrotubation were used to fix the uterine cervix, to facilitate movement of the uterus and to make the pouch of Douglas accessible for inspection. In most cases one or two manipulating cannulae (*Palmer* 1950 and 1963) or one or two measuring needles (*Sjövall* 1963) were inserted through the anterior abdominal wall.

During the years since 1948 many different patterns of laparoscopic instruments have been used at the Department. In earlier years we used the Ruddock laparoscope later on that of Stortz and, sometimes when the ordinary instruments were temporarily at repair the procedure of Sjövall (*Samuelsson* and *Sjövall* 1958 *Sjövall* 1964) Recently we have been very satisfied with the cold light laparoscope of the Drapier Palmer and of the Wolff pattern.

The laparoscopic diagnosis of ovarian endometriosis can be made

a) *directly*

when typical endometrial cysts can be recognized beyond doubt. Then the surface of the cystic enlarged ovary has characteristic areas of scarred whitish tissue containing groups of small gunshotlike dark-red blue or black blisters. Parts of the cyst wall are at times transparent, with a bluish hue The ovary generally adheres to its surroundings by fibrotic tissue very often studded with groups of black endometrial patches as large as pin heads. Spill of the chocolate- or black-coloured contents of the cyst occurring spontaneously or provoked by manipulation is a further *most valuable characteristic*

b) *indirectly*

when the ovary is fixed on the back of the broad ligament and cannot be mobilized from its fixed position. In many such cases endometriotic spots or masses elsewhere in the pelvic peritoneum —the pouch of Douglas on the utero-sacral ligaments etc. give circumstantial *evidence as to the endometrial* nature of the concealed ovarian lesion. Especially in cases where the ovarian surface looks comparatively normal at laparoscopic examination

one has to make sure by manipulating the ovary that it is not fixed on the concealed surface.

The diagnostic accuracy of laparoscopic examination is summarized in Table II

Table II. *Accuracy of Laparoscopic Diagnosis*

Prelaparoscopic Diagnosis	Laparoscopic Diagnosis	
	correct	incorrect
I. Endometriosis	34	2
II. Ovarian tumour		
) ovarian tumour	3	5
b) fibroid or/and ovarian tumour	6	7
	9	12
III. *Acute abdomen*		
) acute salpingitis	3	5
b) acute abdominal pain		2
) ectopic pregnancy	4	–
	7	7
IV. Chronic abdominal pain	3	
V. Sterility	4	4
	57	25

Three cases, all belonging to the group Endometriosis are not listed in the table. Laparoscopy in these cases could not be accomplished for the following reasons. In one case extraperitoneal insufflation and emphysema made further procedures impossible. In two instances the laparoscopist's view was obscured in one case by intestinal loops and in another case by an enlarged pregnant uterus.

The detailed analysis of our material has yielded the following relevant results.

distend the abdominal cavity A volsellum forceps or a cannula for hydrotubation were used to fix the uterine cervix, to facilitate movement of the uterus and to make the pouch of Douglas accessible for inspection. In most cases one or two manipulating cannulae (*Palmer* 1950 and 1963) or one or two measuring needles (*Sjövall* 1963) were inserted through the anterior abdominal wall

During the years since 1948 many different patterns of laparoscopic instruments have been used at the Department. In earlier years we used the Ruddock laparoscope later on that of Stortz and sometimes when the ordinary instruments were temporarily at repair the procedure of Sjövall (*Samuelsson* and *Sjövall* 1958 *Sjövall* 1964) Recently we have been very satisfied with the cold light laparoscope of the Drapier Palmer and of the Wolff pattern.

The laparoscopic diagnosis of ovarian endometriosis can be made

a) *directly*

when typical endometrial cysts can be recognized beyond doubt. Then the surface of the cystic enlarged ovary has characteristic areas of scarred whitish tissue containing groups of small gunshotlike dark red blue or black blisters Parts of the cyst wall are at times transparent with a bluish hue The ovary generally adheres to its surroundings by fibrotic tissue very often studded with groups of black endometrial patches as large as pin-heads Spill of the chocolate- or black-coloured contents of the cyst occurring spontaneously or provoked by manipulation is a further most valuable characteristic.

b) *indirectly*

when the ovary is fixed on the back of the broad ligament and cannot be mobilized from its fixed position. In many such cases endometriotic spots or masses elsewhere in the pelvic peritoneum —the pouch of Douglas on the utero-sacral ligaments etc give circumstantial evidence as to the endometrial nature of the concealed ovarian lesion. Especially in cases where the ovarian surface looks comparatively normal at laparoscopic examination

In one of the five incorrectly diagnosed the final diagnosis was tubo-ovarian cyst. In four cases the laparoscopist concluded his examination having found an ovarian tumour of uncertain nature In three of those cases it is reported at laparotomy that the ovaries adhered to the posterior surface of the uterus and the pouch of Douglas

II b *Prelaparoscopic diagnosis* Fibroid or/and ovarian tumour
13 cases.

Laparoscopic diagnosis

Correct diagnosis	6 (directly)
Incorrect diagnosis	7

Once among the cases incorrectly diagnosed a fibroid was found but the ovaries were judged normal, one ovary however being fixed in the pelvis. Four cases were diagnosed as an ovarian tumour of uncertain nature and in one case an intraligamentary pelvic tumour of the broad ligament was seen. Finally on one occasion the laparoscopic diagnosis was an ovarian tumour probably solid carcinoma. The patient was referred for preoperative radiotherapy and was operated on a fortnight later

Thus in this group the laparoscopist erred in much the same way as in the foregoing group When both groups are combined a correct laparoscopic diagnosis of endometriosis was made in 9 of 21 cases. The laparoscopist often seems to have stopped his examination as soon as he has found some evidence for what could be considered an ovarian tumour of neoplastic origin.

III a. *Prelaparoscopic diagnosis* Acute salpingitis
8 cases

Laparoscopic diagnosis

Correct diagnosis.	3 (directly 2, indirectly 1)
Incorrect diagnosis	5

I	*Prelaparoscopic diagnosis* 36 cases	Endometriosis
	Laparoscopic diagnosis	
	Correct diagnosis	
	directly	28
	indirectly	6
		34
	Incorrect diagnosis	2
		36

In one patient the condition was frankly misjudged. The laparoscopist found no pathological features in the abdominal cavity At laparotomy one month later however both ovaries were found to be fixed by adhesions in the pouch of Douglas the right one containing a chocolate cyst the size of a golf ball The operation was indicated on account of a submucous fibroid seen at hysterography

In the other patient incorrectly diagnosed enlarged ovaries were found at laparoscopy Four days later the patient was operated on for suspected endometriosis the ovaries being fixed by adhesions to the posterior surface of the uterus.

In patients with a prelaparoscopic diagnosis of endometriosis laparoscopy has a fair degree of diagnostic value A firm diagnosis was made in 34 of 36 cases (94 per cent) These cases have thus presented very pronounced and typical characteristics of endometriosis The endoscopist may also have been eager to verify the tentative diagnosis and has therefore made his scrutiny of the pelvic peritoneum and organs with special care and thoroughness

II a.	*Prelaparoscopic diagnosis* 8 cases.	Ovarian tumour
	Laparoscopic diagnosis	
	Correct diagnosis	3 (directly)
	Incorrect diagnosis	5

In one of the five incorrectly diagnosed the final diagnosis was tubo-ovarian cyst. In four cases the laparoscopist concluded his examination having found an ovarian tumour of uncertain nature In three of those cases it is reported at laparotomy that the ovaries adhered to the posterior surface of the uterus and the pouch of Douglas.

II b *Prelaparoscopic diagnosis.* Fibroid or/and ovarian tumour 13 cases.

Laparoscopic diagnosis.

Correct diagnosis	6 (directly)
Incorrect diagnosis	7

Once among the cases incorrectly diagnosed a fibroid was found but the ovaries were judged normal one ovary however being fixed in the pelvis. Four cases were diagnosed as an ovarian tumour of uncertain nature and in one case an intraligamentary pelvic tumour of the broad ligament was seen. Finally on one occasion the laparoscopic diagnosis was an ovarian tumour probably solid carcinoma. The patient was referred for preoperative radiotherapy and was operated on a fortnight later

Thus in this group the laparoscopist erred in much the same way as in the foregoing group. When both groups are combined a correct laparoscopic diagnosis of endometriosis was made in 9 of 21 cases. The laparoscopist often seems to have stopped his examination as soon as he has found some evidence for what could be considered an ovarian tumour of neoplastic origin.

III a Prelaparoscopic diagnosis Acute salpingitis 8 cases.

Lapa oscopic diagnosis.

Correct diagnosis	3 (directly 2, indirectly 1)
Incorrect diagnosis	5

In two of the five incorrectly diagnosed cases the vermiform appendix could not be visualized, the possibility of salpingitis however was excluded. Laparotomy in both cases was performed immediately on the suspicion of an acute appendicitis revealing chocolate cysts in the ovaries In one of those two patients a fixed ovary had been observed by the laparoscopist.

Laparoscopy revealed pelvic peritonitis in three cases

After a delay of four months one of the three patients showing pelvic peritonitis underwent laparotomy The ovary was then found to contain a chocolate cyst which had earlier been implanted in the uterus because of sterility The ovary was bulging out from the implantation site and should have been seen at laparoscopy

The other two patients in which pelvic peritonitis was seen proved at laparotomy to have frankly infected endometriosis

Thus the diagnostic error at laparoscopy in cases where acute salpingitis is suspected may be due to the presence of a concomitant inflammatory condition of the pelvic viscera. This is also the case in one of the patients in the following group

III b *Prelaparoscopic diagnosis* Acute abdominal pain
2 cases

Both were incorrectly diagnosed at laparoscopy In one patient an inflamed epiploic appendage was suspected to be the vermiform appendix at laparoscopy At appendicectomy however infected endometriosis with pelvic peritonitis was also found

The other patient was considered by the laparoscopist to suffer from an ovarian cyst with a twisted stalk and at operation immediately carried out, an endometrial cyst was found.

III c *Prelaparoscopic diagnosis* Ectopic pregnancy
4 cases.

All were correctly diagnosed by the laparoscopist, directly in three patients indirectly in one

IV *Prelaparoscopic diagnosis* Chronic abdominal pain
3 cases

All had the correct laparoscopic diagnosis of endometriosis made directly

V	*Prelaparoscopic diagnosis.* Sterility	
	8 cases	
	Laparoscopic diagnosis	
	Correct diagnosis	4
	Incorrect diagnosis	4

In two patients the correct diagnosis was made directly and in two indirectly on circumstantial evidence by the laparoscopist.

In the four cases erroneously interpreted at laparoscopy two were thought to have chronic salpingitis with adhesions.

In one patient the ovary looked very much enlarged, polycystic, its wall being transparent and was judged to be some kind of multilocular cystoma. Finally in one patient, only adhesions were seen.

To make the correct diagnosis of ovarian endometriosis may be easy enough and in the majority of cases this holds true.

A correct diagnosis was made in 57 of 82 (70 per cent) cases where a laparoscopic examination was possible in our 85 patients suffering from ovarian endometriosis proven by laparotomy and histologic examination.

To many the figure of 70 per cent may appear a poor one regarding the diagnostic efficiency of laparoscopy. When first perusing our material we were also baffled, having had much confidence in the accuracy of laparoscopy as a diagnostic aid.

The detailed analysis of the diagnostic errors leads to the following general conclusions.

1 In some cases ovarian endometriosis is blurred by concomitant inflammatory disease.

2 The laparoscopist, prejudiced and biased by the prelaparoscopic clinical diagnosis, breaks off his laparoscopic examination as soon as he thinks the tentative diagnosis is more or less confirmed.

This seems to have been the case especially when an ovarian tumour of neoplastic type has been initially suspected. In no less than 13 cases the laparoscopist diagnosed some kind of ovarian tumour

3 To exclude the possibility of ovarian endometriosis as far as this is possible at laparoscopy It is absolutely necessary to try to mobilize the ovaries in order to scrutinize both surfaces of each ovary

This has apparently not been done in many examinations and occurred in at least 1/3 of the incorrectly diagnosed cases

By strictly observing the above mentioned points when performing laparoscopy the diagnostic accuracy regarding ovarian endometriosis should be very much improved.

Clinically ovarian endometriosis may appear under the guise of and simulate many different pathological conditions. This is wellknown and also illustrated by the different prelaparoscopic diagnoses in our cases.

The clinical diagnosis of endometriosis was made before laparoscopy in 36 of 82 cases or 44 per cent in the present series At laparoscopy 57 of 82 cases were correctly interpreted as ovarian endometriosis *i e* 70 per cent. Laparoscopy thus has been very much superior to clinical diagnosis only

At laparoscopy endometriosis of the ovary should almost always be considered as a diagnostic possibility when this is not frankly out of the question The laparoscopic examination should be undertaken using every measure to prove or rule out endometriosis One should particularly try to mobilize the ovaries to enable detailed scrutiny If one or both adhere to the posterior surface of the uterus the broad ligament or the pouch of *Douglas* endometriosis should be strongly suspected and further evidence of endometriosis sought. *Typical fibrous tissue containing characteristic* endometrial spots or chocolate -spill provoked by manipulating *the ovary* will then often confirm the suspicion

It is self-evident that a firm diagnosis of endometriosis of the ovary thus avoiding explorative laparotomy is important.

All cases of ovarian endometriosis do not require operative measures.

In many cases where ovarian endometriosis may be suspected either no pathological conditions are found or are such that require special treatment according to the nature of the condition.

In a total of 132 successfully performed laparoscopies on the suspicion of endometriosis during the years 1948–1965 endometriosis was diagnosed in 63 (48 per cent) other pathological conditions were found in 12 (9 per cent) and in no less than 57 (43 per cent) the laparoscopist found nothing abnormal.

SUMMARY

The accuracy of the diagnosis of ovarian endometriosis made by laparoscopy is evaluated. Eighty two cases of gross ovarian endometriosis (Chocolate cysts) previously laparoscoped and verified at laparotomy and by histological examination were investigated. The clinical prelaparoscopic diagnosis was correct in only 36 of 82 cases (44 per cent) The laparoscopic diagnosis was correct in 57 of 82 cases (70 per cent)

The diagnostic errors when performing laparoscopy are analysed. In 36 cases with the clinical prelaparoscopic diagnosis of endometriosis, the laparoscopist made a correct diagnosis in 34 (94 per cent) On the other hand in 46 cases clinically labelled ovarian tumour fibroid or/and ovarian tumour salpingitis acute abdominal pain ectopic pregnancy chronic abdominal pain and sterility laparoscopy revealed the true state of the condition in 23 (50 per cent) only

The diagnostic errors at laparoscopy in cases of ovarian endometriosis are due to

1 In some cases the laparoscopic picture of ovarian endometriosis is blurred by concomitant inflammatory disease.

2 The laparoscopist is not keeping the possibility of ovarian endometriosis in mind at the laparoscopic examination.

3 The laparoscopist is biased by the clinical diagnosis and ceases the laparoscopic examination as soon as the prelaparoscopic diagnosis seems more or less verified.

This seems to have been the case especially when an ovarian tumour of neoplastic type has been initially suspected In no less than 13 cases the laparoscopist diagnosed some kind of ovarian tumour

3 To exclude the possibility of ovarian endometriosis as far as this is possible at laparoscopy it is absolutely necessary to try to mobilize the ovaries in order to scrutinize both surfaces of each ovary

This has apparently not been done in many examinations and occurred in at least 1/3 of the incorrectly diagnosed cases.

By strictly observing the above mentioned points when performing laparoscopy the diagnostic accuracy regarding ovarian endometriosis should be very much improved.

Clinically ovarian endometriosis may appear under the guise of and simulate many different pathological conditions. This is wellknown and also illustrated by the different prelaparoscopic diagnoses in our cases

The clinical diagnosis of endometriosis was made before laparoscopy in 36 of 82 cases or 44 per cent in the present series At laparoscopy 57 of 82 cases were correctly interpreted as ovarian endometriosis i e 70 per cent. Laparoscopy thus has been very much superior to clinical diagnosis only

At laparoscopy endometriosis of the ovary should almost always be considered as a diagnostic possibility when this is not frankly out of the question. The laparoscopic examination should be undertaken using every measure to prove or rule out endometriosis One should particularly try to mobilize the ovaries to enable detailed scrutiny If one or both adhere to the posterior surface of the uterus the broad ligament or the pouch of Douglas endometriosis should be strongly suspected and further evidence of endometriosis sought. Typical fibrous tissue containing characteristic endometrial spots or chocolate -spill provoked by manipulating the ovary will then often confirm the suspicion.

It is self-evident that a firm diagnosis of endometriosis of the ovary thus avoiding explorative laparotomy is important.

All cases of ovarian endometriosis do not require operative measures

Sjövall, Alf and Åkerman, Måns. Acta obst. et gynec. scandinav. 47: 361, 1968.

From the Pathological Institute (Professor C. G. Ahlström) and the Department of Obstetrics and Gynaecology (Professor Alf Sjövall), University Hospital, Lund, Sweden

PERITONEAL GRANULOMAS IN WOMEN DUE TO THE PRESENCE OF ENTEROBIUS S. OXYURIS VERMICULARIS

BY

ALF SJÖVALL AND MÅNS ÅKERMAN

Peritoneal granulomas in women containing the worm Enterobius vermicularis or its ova are rare, almost always incidental findings. They are however interesting from the pathological and differential diagnostic aspects and occasionally produce conditions of clinical importance.

In his excellent comprehensive review "Pathology of Oxyuris" published 1950, *Symmers* paid special reference to peritoneal granulomas analyzing 16 such cases from the literature. He added two of his own cases. Both before and since 1950 a number of additional cases have been reported. We have the opportunity of adding two new cases.

I. Department of Obstetrics and Gynaecology, Lund 5461/66. Housewife, 26 years old. Para III. Between her two last pregnancies she was treated at home for oophoritis.

One and a half years before admission she complained of pain in the back and the groins, dysmenorrhoea and dyspareunia.

The uterus was retroflexed, severly tender in the posterior fornix especially at the right side. Anteflexion was possible but caused pain. In other respects everything was normal at bimanual examination. The ovaries felt normal. Hysterosalpingography was normal.

On account of her dyspareunia and dysmenorrhoea, presacral resection of the hypogastric plexus, bilateral partial lumbosacral sympathectomy and antefixation of the uterus according to Baldy-Webster was performed (*Sjövall*).

At the right uterosacral ligament the peritoneal surface presented ... small

4 The laparoscopist does not use all technical facilities especially trying to manipulate the ovaries If the ovary is fixed to the posterior surface of the uterus the broad ligament or in the pouch of Douglas ovarian endometriosis should be strongly suspected and further evidence sought.

REFERENCES

Palmer R. La stérilité involontaire. Paris 1950

– Les explorations fonctionelles gynécologiques. Paris 1963

Samuelsson S and *Sjövall A.* Förh Nord. Fören. Obst. Gynek. 10 möte i Stockholm 1958 Lund 1959 p 114

Sjövall A. Acta obst. et gynec. scandinav *42* 279 1963

– Proceedings of the first international symposium on gynecological celioscopy Palermo 1964 p 77–80

Received on July 12, 1967



Fig 2 Case II Granulomas on the surface of the ovary ×11.5.



Fig 3 Case II Partially calcified ovarian granuloma containing Enterobius [illegible] in its necrotic centre ×100



Fig 1 Case I. Enterobius ova surrounded by granulomatous tissue ×225.

area 1×1 1/2 cm, covered by greyish-red granulomatous tissue On the adjacent surface of the right ovary identical tissue of exactly the same extension was found This was the only lesion to be seen in the peritoneal cavity There were no other gns of inflammatory disease of the peritoneum or the pelvi organs The fimbriated ends of the tubes were open.

Histological examination (*Akerman*) showed granulomatous tissue containing numerous eosinophil leucocytes and typical Enterobius ova (Fig 1)

On the check up about one month after the operation the patient was well and was symptom free When questioned she said that she had suffered from pinworms earlier

II Department of Obstetrics and Gynaecology Hälsingborg Housewife 38 years old. Para 0 Curettings in 1955 and 1958 for abnormal bleeding yielded a normal endometrium. On the latter occasion the left ovary was found to be enlarged to the size of a golf ball. She did not complain of any pain. At laparotomy (*Möller*) part of one ovary containing a cyst was resected

Histological examination (*Ahlström*) Some cystic follicles and in a circumscribed area on the ovarian surface small granulomas surrounded by connective tissue infiltrated by many eosinophil leucocytes The centre of the granulomas consisted of an amorphous eosinophilic substance containing Enterobius ova (Figs. and 3)

the abdominal cavity One of the worms showed vigorous move ments. The intestine including the vermiform appendix showed no perforation. The peritoneum of the lower ileum and in the pouch of Douglas was reddened (*Maier* 1956) The worms may have penetrated the gut actively

Vuillemin (1902) held that a perineal abscess observed by *Froelich* (1897) containing more than 60 female pinworms is evidence for penetration of the intestinal wall by the worms. This seems rather dubious.

In a series of Douglascopic colour photographies *Menken* (1953) watched an oxyuris vermicularis from the very perfora tion of the vaginal vault until it settled and was incapsulated by the peritoneum The short abstract of this congress communica tion does not contain the pictures.

Haematogenic spread of pinworms may occur This is evident from findings in the liver (*Slais* 1962 *Linell* 1967) and the spleen (*Nathan* 1928)

However pinworm granulomas of the peritoneum in women arise after the parasite has reached the peritoneal cavity through the genital tract. Penetration of the intestine or the vaginal vault seem improbable routes of invasion in these cases.

The transport of pinworms through the uterus and the tubes can be explained by its own activity which, however may be enhanced by retrograde movements of the uterine and tubal muscular tissue. Such movements occur Well-known to any pelvic surgeon is the phenomenon of retrograde bleeding from the fimbriated ends of the tubes into the abdominal cavity during menstruation (*Müller* 1925 *Ottow* 1936 and others)

Furthermore fragments of endometrial tissue have been found in the tubal lumen (*Neumann* 1930 *Baer* 1932, and others)

These observations—which support *Sampson's* implantation theory of the aetiology of external endometriosis—are also in keeping with the ascension of Enterobius-worms and possibly their ova only from the uterine to the abdominal cavity

The propagation of Enterobius through the lumen of the ge- nital tract is supported by the following facts

1) Peritoneal pinworm granulomas are found only in women.
2) The granulomas contain only female pinworms or ova. The

In most reported cases of granulomas as in our two cases only Enterobius ova, solitary or more often in groups have been present. In quite a number of cases intact female pinworms or parts of worms in dissolution were found. It thus seems that it is the worm proper which chiefly gives rise to the granulomatous reaction. The ova resist the destructive power of the host and remain intact as comparatively inert bodies representing lasting evidence of the earlier presence of worms.

There are two possible routes for the spread of pinworms to the abdominal cavity

I Tissue penetration of the intestinal wall or the vaginal vault perhaps permitting further propagation, lymphogenic or haematogenic.

II Canalicular transportation through the lumen of the genital tract.

Several cases reported give conclusive evidence regarding the capacity of pinworms to penetrate damaged or undamaged tissue It is of course always difficult to decide whether the damage is entirely due to the worm or has been present beforehand facilitating the worm s propagation.

It is self-evident that pinworms can enter the abdominal cavity at perforation of the intestine e g from a perforated inflamed vermiform appendix In the wall of the appendix Enterobius granulomas were seen by *Stark* (1958) and others We will pass over the intricate question regarding Appendicitis ex Oxyure and Appendicopathia Oxyurica (*Unterberger* 1908 *Ashoff* 1913 *Rheindorf* 1920 and others)

The case of *Bijlmer* (1946) is an exceptional example proving tissue penetration by pinworms

At the autopsy performed on a man who died of ulcerative enterocolitis tens of thousands larvae of Enterobius male ones as well as female ones in different stages of evolution were found in all layers of the intestinal wall Blood vessels as well as lymphatics were invaded Probably the invasion of worms was secondary to the inflammatory disease of the gut.

In a man 71 years old laparotomized because of acute abdominal pain two specimens of Enterobius were found free in

the abdominal cavity One of the worms showed vigorous movements The intestine including the vermiform appendix showed no perforation. The peritoneum of the lower ileum and in the pouch of Douglas was reddened (*Maier* 1956) The worms may have penetrated the gut actively

Vuillemin (1902) held that a perineal abscess observed by *Froelich* (1897) containing more than 60 female pinworms is evidence for penetration of the intestinal wall by the worms. This seems rather dubious

In a series of Douglascopic colour photographies *Menken* (1953) watched an oxyuris vermicularis from the very perforation of the vaginal vault until it settled and was incapsulated by the peritoneum The short abstract of this congress communication does not contain the pictures.

Haematogenic spread of pinworms may occur This is evident from findings in the liver (*Slais* 1962 *Linell* 1967) and the spleen (*Nathan* 1928)

However pinworm granulomas of the peritoneum in women arise after the parasite has reached the peritoneal cavity through the genital tract. Penetration of the intestine or the vaginal vault seem improbable routes of invasion in these cases.

The transport of pinworms through the uterus and the tubes can be explained by its own activity which, however may be enhanced by retrograde movements of the uterine and tubal muscular tissue. Such movements occur Well-known to any pelvic surgeon is the phenomenon of retrograde bleeding from the fimbriated ends of the tubes into the abdominal cavity during menstruation (*Müller* 1925 *Ottow* 1936 and others)

Furthermore fragments of endometrial tissue have been found in the tubal lumen (*Neumann* 1930 *Baer* 1932, and others)

These observations—which support *Sampson*'s implantation theory of the aetiology of external endometriosis—are also in keeping with the ascension of Enterobius-worms and possibly their ova only from the uterine to the abdominal cavity

The propagation of Enterobius through the lumen of the genital tract is supported by the following facts

1) Peritoneal pinworm granulomas are found only in women.
2) The granulomas contain only female pinworms or ova. The

females emigrate from the rectum to the anal region the males remain in the intestine From the perianal skin, female pinworms may easily enter the vagina

Swellengrebel and *Sterman* (1961) hold that the female genital tract is invaded by larvae hatched on the perineum considering the short life span of gravid female worms

3) Adult female pinworms were seen in the vagina by *Vaughan* (1880) *Spitzer* (1892) *Heller* (1903) *Linell* (1967) found a fine specimen in the Bartholin gland. In an early squamous epithelial carcinoma a granuloma containing a female Enterobius and ova is reported by *Klee* (1920)

4) Inspecting the vagina *Simons* (1899) held what he recognized as being a 15 mm female pinworm disappear into the cervix. After a minute s delay he scoped out a second smaller worm *Bobeck* (1967) observed a 10 mm female pinworm creeping out from the external os

5) In the endometrium of the uterine corpus granulomas containing female pinworms and ova are reported by *Nairn* and *Duguid* (1954) and *Schenken* and *Tamisiea* (1956)

6) Between the isthmus and the ampulla of a normal Fallopian tube *Tschamer* (1919) observed two vigorously agile female pinworms In the folds of the tubal mucosa *Leschke* (1951) found a calcified female pinworm containing ova Ova were also found in the submucosa There was no tissue reaction. The specimen was obtained from a patient operated on for fibroids and endometriosis Granulomas containing worms or ova were comparatively often seen in acutely or chronically inflamed tubes *Chiari* *1928 Jones* and *Bunting, 1931 Ku* and *Wu 1934 1935 Paulik* 1940 *Marquardt* 1943 *Hartl* 1948 *Croce et al* 1956 *Chevrel et al* 1962

7) Pinworm granulomas are almost always confined to the genital tract, the pelvic peritoneum hernine or the greater omentum

Considering the common occurrence of pinworm infestation of the gut it may seem astonishing that the abdominal cavity of women seldom is invaded by the worms Protecting mechanisms such as the acid reaction of the vaginal fluid and the mucous plug in the cervix may play a part (*Marquardt* 1943 and

others) The motor function of the muscular tissue of the genital tract, normally directed outwards may also be of importance. These protecting properties as is well known vary during different ages and states of health and are sometimes disturbed.

The distribution and site of pinworm-granulomas on the peritoneum is varied. Generally the individual foci are as small as pin-heads or peas exceptionally bigger reaching the size of a walnut. Sometimes they are solitary sometimes multiple and may then be spread over a comparatively large area. According to the age of the condition, they are soft, disintegrating, callous or hard as stone. The colour is reddish-grey greyish or yellowish.

On histological examination the granulomas have the following characteristics

In early granulomas around a centre containing ova remains of the worm or comparatively well preserved worms there is a loose connective tissue densely infiltrated by lymphocytes Eosinophil leucocytes are often abundant. At times giant cells are present. In the course of time the granulomas become gradually encapsulated by fibrous tissue The centre of the disintegrated worm or the ova becomes necrotic. Charcot Leyden crystals may be present The granulomas are often more or less calcified.

The principal localizations of granulomas are

The fimbriated end of the tube
Marro (1901) Chomet (1942); *Köher* (1955)

Multiple foci on the serous membrane of the tube
Mallory (1938)

The tube and the ovary
Arthur and Tomlinson (1958) Cherrel *et al* (1962); Case I.

The ovary only
Gill and Smith (1952) Lemmon et al (1960) our own Case II

The mesosalpinx
Symmers (1950) Case I

The surface of the uterus
a) solitary *Bormann* (1934) *Fatherree et al.* (1951), *Sleb* (1962)
b) multiple *Hendrlock* (1926) *Valterio* (1940); *Symmers* (1950) Case I.

females emigrate from the rectum to the anal region the males remain in the intestine. From the perianal skin, female pinworms may easily enter the vagina

Swellengrebel and *Sterman* (1961) hold that the female genital tract is invaded by larvae hatched on the perineum considering the short life span of gravid female worms

3) Adult female pinworms were seen in the vagina by *Vaughan* (1880) *Spitzer* (1892) *Heller* (1903) *Linell* (1967) found a fine specimen in the Bartholin gland. In an early squamous epi thelial carcinoma a granuloma containing a female Enterobius and ova is reported by *Klee* (1920)

4) Inspecting the vagina *Simons* (1899) held what he recognized as being a 15 mm female pinworm disappear into the cervix. After a minute s delay he scoped out a second smaller worm *Bobeck* (1967) observed a 10 mm female pinworm creeping out from the external os

5) In the endometrium of the uterine corpus granulomas containing female pinworms and ova are reported by *Nairn* and *Duguid* (1954) and *Schenken* and *Tamisiea* (1956)

6) Between the isthmus and the ampulla of a normal Fallopian tube *Tschamer* (1919) observed two vigorously agile female pinworms In the folds of the tubal mucosa *Leschke* (1951) found a calcified female pinworm containing ova Ova were also found in the submucosa There was no tissue-reaction. The specimen was obtained from a patient operated on for fibroids and endometriosis Granulomas containing worms or ova were comparatively often seen in acutely or chronically inflamed tubes *Chiari* 1928 *Jones* and *Bunting*, 1931 *Ku* and *Wu* 1934 1935 *Pawlik* 1940 *Marquardt* 1943 *Hartl* 1948 *Croce et al.* 1956 *Chevrel et al* 1962

7) Pinworm granulomas are almost always confined to the genital tract the pelvic peritoneum herniae or the greater omentum

Considering the common occurrence of pinworm infestation of the gut it may seem astonishing that the abdominal cavity of women seldom is invaded by the worms Protecting mechanisms such as the acid reaction of the vaginal fluid and the mucous plug in the cervix may play a part (*Marquardt* 1943 and

Schmutzler (1957) where multiple granulomas were spread on the peritoneum the symptoms were probably due to the Enterobius infestation. The case reported by *Beddoe* (1956) is rather convincing. A girl, 16 months old, was taken ill with acute abdominal pain, fever and constipation. At laparotomy a mass obliterating the pouch of Douglas was found. The mass looked like malignant neoplastic tissue or inflammatory tissue. A biopsy specimen removed from an adhesion between the sigmoid colon and the pouch of Douglas contained a female Enterobius and numerous ova.

Acute abdominal symptoms which are never elucidated even at laparotomy are of such common occurrence that one should be very cautious regarding the pathogenetic significance of pinworm granulomas and their capacity to cause substantial illness. In this connection it is to be remembered how often appendicectomy on the suspicion of appendicitis in women only reveals the finding of a normal appendix and the patient recovers as soon as the immediate inconveniance caused by the intervention has passed. It should also be repeated that most granulomas are incidental findings without clinical importance.

Although this paper is limited to comprise peritoneal granulomas it should be mentioned that it is difficult to decide whether Enterobius infestation of the tubes can give rise to salpingitis or whether the worms have settled in a previously inflamed tube. In the cases reported by Chiari (1928), *Jones* and *Bunting* (1931), *Marquardt* (1943) and *Croce et al.* (1956) the salpingitis was probably due to the worm.

Enterobius granulomas encountered at laparotomy are of importance in diagnostic respects. To the naked eye they are similar to diverse other lesions. The surgeon suspected malignancy in the cases reported by *Butter* (1929) and Beddoe (1956). The granuloma in the case of *Hendrlock* (1926) looked like a fibroid. In the case of Peter (1957) a granuloma of the greater omentum was interpreted as being a tuberculous lymphoma.

In some cases of disseminated granulomas, peritoneal tuberculosis was suspected (*Goodale* and *Krischner* 1930; *Valteris* 1940). In one case the surgeon felt convinced of the diagnosis of tuberculosis. The patient was already sent to a sanatorium be-

The round ligament:
Deeds (1947)

The utero-sacral ligament
Freudenberg (1951)

The vesico-uterine fold:
Frorlep (1938)

The pouch of Douglas.
a) solitary: *Schneider* (1904) *Unterberger* (1908) *Butter* (1929) *Stark* (1958) *Chevrel et al* (1962) Case III, our own Case I.
b) multiple: *Kolb* (1902) *Strada* (1907) *Schmincke* (1930) *Helm* (1954)

Disseminated widely spread on the pelvic peritoneum.
Wegner (1933) *Pawlick* (1940) case I *Brenner* (1952) *Schenken* and *Tamislea* (1956) *Beddoe* (1956) *Hübner* (1957)

The recto-vaginal septum.
Kecht (1933) *Huhn* (1963)

The colon and mesocolon
Kaufmann (1922)

The small intestine
Goodale and *Krischner* (1930) *Schmutzler* (1957)

The great omentum.
Bodechtel (1927) *Schmincke* (1930) *Schneider* (1931) *Wegner* (1933) *Hübner* (1957) Cases I–III *Peter* (1957) *Stark* (1958) case IV *Leigh* and *Saylor* (1964)

Processus vaginalis of the peritoneum.
Fi gerland and *Meridiek* (1941)

Inguinal herniae
Ebbehoj (1961) *Karnauchow* (1961) *Slais* (1962) Case IV

As a rule Enterobius granulomas are without clinical importance and are incidental findings In some cases however they seem to have been the main cause for surgical intervention.

This occurred comparatively often in cases of granulomas of the greater omentum where the symptoms and signs were those of an acute appendicitis but the appendix was normal (*Schmincke* 1930 *Schneider* 1931 *Wegner* 1933 *Hubner* 1957 Cases I–III *Peter* 1957 *Stark* 1958 Case IV *Leigh* and *Saylor* 1964)

In the cases reported by *Mallory* (1938) *Brenner* (1952) and

Schmutzler (1957) where multiple granulomas were spread on the peritoneum the symptoms were probably due to the Enterobius infestation. The case reported by *Beddoe* (1956) is rather convincing. A girl 16 months old, was taken ill with acute abdominal pain, fever and constipation. At laparotomy a mass obliterating the pouch of Douglas was found. The mass looked like malignant neoplastic tissue or inflammatory tissue. A biopsy specimen removed from an adhesion between the sigmoid colon and the pouch of Douglas contained a female Enterobius and numerous ova.

Acute abdominal symptoms which are never elucidated even at laparotomy are of such common occurrence that one should be very cautious regarding the pathogenetic significance of pin worm granulomas and their capacity to cause substantial illness. In this connection it is to be remembered how often appendic ectomy on the suspicion of appendicitis in women only reveals the finding of a normal appendix and the patient recovers as soon as the immediate inconveniance caused by the intervention has passed. It should also be repeated that most granulomas are incidental findings without clinical importance.

Although this paper is limited to comprise peritoneal granulomas it should be mentioned that it is difficult to decide whether Enterobius infestation of the tubes can give rise to salpingitis or whether the worms have settled in a previously inflamed tube. In the cases reported by *Chiari* (1928) *Jones* and *Bunting* (1931) *Marquardt* (1943) and *Croce et al.* (1956) the salpingitis was probably due to the worm.

Enterobius granulomas encountered at laparotomy are of importance in diagnostic respects To the naked eye they are similar to diverse other lesions. The surgeon suspected malignancy in the cases reported by *Butter* (1929) and *Beddoe* (1956) The granuloma in the case of *Hendrick* (1926) looked like a fibroid. In the case of *Peter* (1957) a granuloma of the greater omentum was interpreted as being a tuberculous lymphoma.

In some cases of disseminated granulomas peritoneal tuberculosis was suspected (*Goodale* and *Krischner* 1930 *Valteris* 1940) In one case the surgeon felt convinced of the diagnosis of tuberculosis. The patient was already sent to a sanatorium be-

The round ligament.
Deeds (1947)

The utero-sacral ligament
Freudenberg (1951)

The vesico-uterine fold.
Froriep (1938)

The pouch of Douglas.
a) solitary *Schneider* (1904) *Unterberger* (1905) *Butter* (1929) *Stark* (1958) *Chevrel et al* (1962) Case III our own Case I
b) multiple: *Kolb* (1902) *Strada* (1907) *Schmincke* (1930) *Heim* (1954)

Disseminated widely spread on the pelvic peritoneum.
Wegner (1933) *Pawlick* (1940) case I *Brenner* (1952) *Schenken* and *Tamisiea* (1956) *Beddoe* (1956) *Hübner* (1957)

The recto-vaginal septum.
Kecht (1933) *Huhn* (1963)

The colon and mesocolon
Kaufmann (1922)

The small intestine
Goodale and *Krischner* (1930) *Schmutzler* (1957)

The great omentum.
Bodechtel (1927) *Schmincke* (1930) *Schneider* (1931) *Wegner* (1933) *Hübner* (1957) Cases I–III *Peter* (1957) *Stark* (1958) case IV *Leigh* and *Saylor* (1964)

Processus vaginalis of the peritoneum.
Fingerland and *Maršalek* (1941)

Inguinal herniae
Ebbehoj (1961) *Karnauchow* (1961) *Slais* (1962) Case IV

As a rule Enterobius granulomas are without clinical importance and are incidental findings In some cases however they seem to have been the main cause for surgical intervention.

This occurred comparatively often in cases of granulomas of the greater omentum where the symptoms and signs were those of an acute appendicitis but the appendix was normal (*Schmincke* 1930 *Schneider* 1931 *Wegner* 1933 *Hubner* 1957 Cases I–III *Peter* 1957 *Stark* 1958 Case IV *Leigh* and *Saylor* 1964)

In the cases reported by *Mallory* (1938) *Brenner* (1952) and

Chomet B Arch. Path. 34 742, 1942
Croce E J et al New England J Med. 254 67 1956
Deeds D D Am. J Obst. & Gynec. 54 890 1947
Ebbehøj J Ugesk. laeger 123 308 1961
Fatherree J P et al Mississippi Doctor 29 159 1951
Fingerland A. and Markalous J Casop. lék. česk. 80 532, 1941
Freudenberg, H Geburtsh. u. Frauenh. 11 848 1951
Fromp E Zentralbl. Gynäk. 62 923 1938
Gill A J and Smith A L Am. J Clin. Path. 22 879 1952
Goodale R H and Krischner H Arch. Path 9 631 1930
Hartl H Zentralbl. Gynäk. 70 816 1948
Heim K Zentralbl. Gynäk. 76 1359 1954
Heller A Deutsches Arch. klin. Med 77 21 1903
Hendrock Zentralbl. Gynäk. 50 3274 1926
Hueter C Zentralbl. allg Path. 30 675 1919–1920
Ibidem 31 432, 1920–1921
Huhn F O Geburtsh. u Frauenh. 23 627 1963
Hübner O Deutsche med. Wchnschr 82 743 1957
Jones W J and Bunting, C. H Arch. Path. 11 229 1931
Karmanchow P N Canad. M A. J 84 388 1961
Kastranek F Wien klin. Wchnschr 60 51 1948
Kaufmann E Lehrbuch der speziellen pathologischen Anatomie für Studierende und Aerzte Ed. 7–8 Berlin und Leipzig (W de Gruyter & Co.) 1922, p 672
Kecht B Wien med. Wchnschr 83 581 1933
Klee F Zentralbl Gynäk 44 939 1920
Kolb R Centralbl Bakt (Orig.) 31 268 1902
K D Y T Far East A. Trop Med 1 605 1934
Köhler H Geburtsh u Frauenh. 15 749 1955
Lautman H H et al Am J Obst & Gynec 79 1178, 1960
Leck G Deutsche med Wchnschr 74 1507 1949
Leigh F D and Saylor H L S Dakota J Med. Pharm. 17 25 1964
Leschke H Zentralbl allg Path 87 355 1951
Lowell F Personal communication 1967
Maier F Klin Med 11 319 1956
Mallory T B New England J Med 218 305 1938
Marquardt S Zentralbl Gynäk 67 612, 1943
Marro G Arch per le sc med 25 161 1901
Gior d Accad di med di Torino 7 231 1901
Meukers Geburtsh. Frauenh 13 950 1953
Müller P Zentralbl Gynäk. 49 1977 1925
Nairn R C and Dugeid H L D J Clin. Path. 7 228 1954
Nathan H Frankfurt Ztschr Path. 36 82, 1928
Neumann H O Arch Gynäk 139 358 1930
Ottow Zentralbl Gynäk 60 2962 1936

fore the result of a biopsy revealed the correct diagnosis (*Schmutzler* 1957) A patient, 35 years old, underwent bilateral oophorectomy (?) on the naked eye-diagnosis of tubal and ovarian tuberculosis (*Chevrel et al* 1962 Case I)

At histological examination which is the only means to obtain a firm diagnosis of Enterobiasis causing peritoneal granuloma, the differential diagnosis regarding other worms and their ova must be considered.

SUMMARY

Two cases of Enterobius granulomas in the abdominal cavity are reported. One was located on the pelvic peritoneum, the other on the ovary Cases of peritoneal pinworm granulomas from the literature are surveyed regarding the transport of the worm to the abdominal cavity the different sites of the granulomas their possible clinical significance and their differential diagnostic importance

Acknowledgement

We thank Tore Wahlen, M.D., head of the department of obstetrics and gynaecology Halsingborg, for permission to publish Case II

REFERENCES

Arthur H R. and *Tomlinson B E* J Obst & Gynaec. Brit. Emp 65 995 1958
Ashoff L. Med Klin 9 249 1913
Baer W Zentralbl Gynäk 56 3106 1932
Beddoe H L Am. J Dis Children 91 577 1956
Bijlmer J J Parasitol 32 359 1946
Bobeck S Personal communication 1957
Bodechtel G Zentralbl Gynäk 51 1500 1927
Borrmann F Zentralbl allg Path 60 305 1934
Brandt M Verh. deutsch Gesellsch Path 33 Tagung 1949 Stuttgart 1950 p 180
Brenner M Geburtsh u Frauenh 12 763 195
Butter R Ueber Oxyureneier in einem Serosaknötchen des Douglas'schen Raumes Diss München (Rudolf Müller & Steinicke) 1929
Chevrel M L et al Arch. d anat path 10 255 1962
Chiari Prag med Wchnschr 27 227 1902
Chiari H Arch path Anat 269 730 1928

Chomet B., Arch. Path. 34 742, 1942
Croce E J *et al.* New England J Med. 254 67 1956
Dereck D D Am. J Obst. & Gynec. 54 890 1947
Ebbehøj J Ugeskr. laeger 123 308 1951
Fatherree J P *et al.* Mississippi Doctor 29 159 1951
Fingerland, A. and Merhaut, J Casop lék. česk. 80 532 1941
Freudenberg, H Geburtsh. u. Frauenh. 11 848 1951
Frommolt E Zentralbl. Gynäk. 62 923 1938
Gill A. J and Smith A. L Am. J Clin. Path. 22 879 1952
Goodale R H and Kretschmer H Arch. Path. 9 631 1930
Hartl H Zentralbl. Gynäk. 70 816 1948
Hauser K. Zentralbl. Gynäk 76 1359 1954
Heller A Deutsches Arch. klin. Med. 77 21 1903
Hendriock, Zentralbl. Gynäk 50 3274 1926
Hueter C Zentralbl allg Path 30 675 1919–1920
Ibidem 31 432, 1920–1921
Huber F O Geburtsh u. Frauenh 23 827 1963
Hübner O Deutsche med Wchnschr 82 743 1957
Jones W J and Brunning, C. H Arch. Path. 11 229 1931
Karanauchow *P N* Canad M A. J 84 388 1961
Kastranek F Wien klin Wchnschr 60 51 1948
Kaufmann E. Lehrbuch der speziellen pathologischen Anatomie für Studierende und Aerzte Ed 7–8 Berlin und Leipzig (W de Gruyter & Co) 1922, p 672
Kecht B Wien med. Wchnschr 83 581 1933
Klee F Zentralbl Gynäk 44 939 1920
Kolb R Centralbl Bakt (Orig) 31 268 1902
Ku D Y T Far East A Trop Med, 1 605 1934
Köhler H Geburtsh u. Frauenh. 15 749 1955
Lockwood, H H *et al* Am J Obst & Gynec. 79 1178, 1960
Leuck G Deutsche med Wchnschr 74 1507 1949
Leigh F D and *Saylor H L* S. Dakota J Med. Pharm. 17 25 1964
Leschke H Zentralbl allg Path. 87 385 1951
Lowell F Personal communication 1967
Maier Fr Klin. Med 11 319 1956
Mallory T B New England J Med. 218 305 1938
Marquardt S Zentralbl. Gynäk 67 612, 1943
Marro G Arch per le sc. med. 25 161 1901
Gior d. Accad di med di Torino 7 251 1901
Merhaut Geburtsh. u Frauenh. 13 950 1953
Müller P Zentralbl. Gynäk. 49 1977 1925
Nairn R. C and *Dugald H L D* J Clin. Path. 7 228 1954
Nathan H Frankfurt. Ztschr Path. 36 82, 1928
Neumann H O Arch. Gynäk. 139 358, 1930
Ottow Zentralbl. Gynäk. 60 2862, 1936

fore the result of a biopsy revealed the correct diagnosis (*Schmutzler* 1957) A patient 35 years old, underwent bilateral oophorectomy (!) on the naked eye-diagnosis of tubal and ovarian tuberculosis (*Chevrel et al* 1962 Case I)

At histological examination which is the only means to obtain a firm diagnosis of Enterobiasis causing peritoneal granuloma, the differential diagnosis regarding other worms and their ova must be considered.

SUMMARY

Two cases of Enterobius granulomas in the abdominal cavity are reported One was located on the pelvic peritoneum the other on the ovary Cases of peritoneal pinworm granulomas from the literature are surveyed regarding the transport of the worm to the abdominal cavity the different sites of the granulomas their possible clinical significance and their differential diagnostic importance

Acknowledgement

We thank Tore Wahlén M D head of the department of obstetrics and gynaecology Hälsingborg, for permission to publish Case II

REFERENCES

Arthur H R. and *Tomlinson B. E* J Obst. & Gynaec Brit Emp *65* 995 1958
Ashoff L Med. Klin *9* 249 1913
Baer W Zentralbl. Gynäk. *56* 3106 1932
Beddoe H L Am J Dis Children *91* 577 1956
Bijlmer J J Parasitol. *32* 359 1946
Bobeck S Personal communication 1957
Bodechtel G Zentralbl. Gynäk *51* 1500 1927
Borrmann F Zentralbl allg Path. *60* 305 1934
Brandt M Verh. deutsch. Gesellsch. Path 33 Tagung 1949 Stuttgart 1950 p 180
Brenner M Geburtsh. u. Frauenh *12* 763 1952
Butter R Ueber Oxyureneier in einem Serosaknötchen des Douglas'schen Raumes Diss München (Rudolf Müller & Steinicke) 1929
Chevrel M L et al. Arch. d anat. path. *10* 255 1962
Chiari Prag med. Wchnschr *27* 227 1902
Chiari H Arch. path. Anat. *269* 730 1928

Danielsson, Mats, Engfeldt, Bengt, Fors, Bertil, Larsson, Börje and Naeslund, John, Acta obst. et gynec. scandinav. 47 373, 1968

From the Institute of Pathology II (Prof. Bengt Engfeldt) and the Department of Radiobiology, the Gustaf Werner Institute (Assoc. Prof. Börje Larsson), University of Uppsala, Uppsala, Sweden

EFFECT OF HIGH ENERGY PROTONS ON V×2 CARCINOMA IMPLANTED IN THE LOWER ABDOMINAL WALL OF THE RABBIT

Comparison between single and fractionated irradiation schemes

BY

MATS DANIELSSON, BENGT ENGFELDT, BERTIL FORS, BÖRJE LARSSON AND JOHN NAESLUND

Introduction

In earlier investigations on the effect of high energy protons on V×2 carcinoma, superficial tumours in the rabbit ear (*Naeslund et al.* 1958, *Falkmer et al.* 1959) as well as deep-lying tumours (*Naeslund et al.* 1959) were studied. As regards this type of carcinoma it should be noted that superficial tumours may show spontaneous regression while tumours implanted in muscle or in abdominal organs invariably proliferate and kill the host by infiltration of the surrounding tissues and the rapid dissemination of metastases.

The aim of the present investigation was to study the reactions of malignant growth to proton irradiation with special attention to the pelvic organs. Well nutriated tissue was chosen as the site of tumour implantation; the anterior abdominal wall was selected because the operation could be performed here much easier and with fewer side-effects than on implantation in the pelvic organs.

It is evident from the results of an earlier investigation (*Naeslund et al.* 1959) that protons applied under the conditions of the present study and in a single dose of 3000–4000 rad may cause complete regression of V×2 carcinoma. Similar observations have been made on human squamous cell carcinoma of the uterine

Pawlick H Arch. Gynäk. *170* 342, 1940
Peter H Zentralbl. Gynäk. 79 1616 1957
Rheindorf A. Die Wurmfortsatzentzündung. Eine pathogenetische und histologische Studie mit besonderer Berücksichtigung der Bedeutung der Helminthen speziell der Oxyuren und wichtiger allgemeiner klinischer Gesichtspunkte. Berlin (S Karger) 1920
Schenken J R. and *Tamisiea J* Arch Surg 73 309 1956
– Am. J Obst. & Gynec. 72 913 1956
Schmincke München. med. Wchnschr 77 1741 1930
Schmutzler R. Ztschr ges. inn. Med. *12* 804 1957
Schneider Fr Zentralbl. Chir 58 1301 1931
Schneider P Centralbl Bakt. 36 550 1904
Schröder R. Lehrbuch der Gynäkologie für Studierende und Aerzte Leipzig (F C. W Vogel) 1922, p 344
Simons E. M Centralbl. Gynäk. 23 777 1899
Slais J Zentralbl allg Path. *103* 214 1962
Smith W S and *Denton J* Am. J Obst. & Gynec. *16* 205 1928
Spitzer B B. R. Wien. med. Wchnschr 42 6 1892
Stark L Med. Klin. 53 2017 1958
Strada F Arch per le sc. med *31* 418 1907
Strassen O Ueber den Befund von Oxyuris vermicularis im weiblichen Genitaltraktus mit einem neuen Fall aus dem pathologischen Institut zu Bonn. Diss Bonn 1915
Swellengrebel N H and *Sterman M M* Animal parasites in man. Princeton 1961
Symmers W St C Arch. Path. *50* 475 1950
Tschamer F Zentralbl Gynäk *43* 959 1919
Unterberger F Centralbl Bakt. *47* 495 1908
Valteris K. Medicina Kaunas *21* 293 1940
Vaughan R. S St Louis Clin. Rec 7 271 1880–1881
Wegner H Peritonitis oxyurica mit Bekanntgabe eines akuten klinische Erscheinungen machenden Falles Diss Heidelberg 1933
Vix E Ueber Entozoën bei Geisteskranken insbesondere über die Bedeutung das Vorkommen und die Behandlung von Oxyuris vermicularis. Berlin (A. Hirschwald) 1860
Wu L. C Chinese M. J *49* 256 1935
Vuillemin P Centralbl. (Orig) *32* 358 1902

Received on July 12, 1967

Danielsson, Mats, Engfeldt, Bengt, Fors, Bertil, Larsson Börje and Naeslund, John, Acta obst. et gynec. scandinav. 47 373, [illegible]

From the Institute of Pathology II (Prof Bengt Engfeldt) and the Department of Radiobiology the Gustaf Werner Institute (Assoc Prof Börje Larsson) University of Uppsala Uppsala Sweden

EFFECT OF HIGH-ENERGY PROTONS ON V×2 CARCINOMA IMPLANTED IN THE LOWER ABDOMINAL WALL OF THE RABBIT

Comparison between single and fractionated irradiation schemes

BY

MATS DANIELSSON BENGT ENGFELDT BERTIL FORS, BÖRJE LARSSON AND JOHN NAESLUND

Introduction

In earlier investigations on the effect of high energy protons on V×2 carcinoma, superficial tumours in the rabbit ear (*Naeslund et al* 1958 *Falkmer et al.,* 1959) as well as deep-lying tumours (*Naeslund et al.* 1959) were studied. As regards this type of carcinoma it should be noted that superficial tumours may show spontaneous regression while tumours implanted in muscle or in abdominal organs invariably proliferate and kill the host by infiltration of the surrounding tissues and the rapid dissemination of metastases.

The aim of the present investigation was to study the reactions of malignant growth to proton irradiation with special attention to the pelvic organs. Well nutriated tissue was chosen as the site of tumour implantation the anterior abdominal wall was selected because the operation could be performed here much easier and with fewer side-effects than on implantation in the pelvic organs.

It is evident from the results of an earlier investigation (*Naeslund et al* 1959) that protons applied under the conditions of the present study and in a single dose of 3000–4000 rad may cause complete regression of V×2 carcinoma. Similar observations have been made on human squamous cell carcinoma of the uterine

Table I Plan of the Experiment (cf Fig 3).

Group No	Number of Animals	Tumour Implanted	Dose in Rad of Radiation Seance No. 1	2	3	4	5	Total Dose (Rad)
I	10	No	3000	—	—		—	3000
II	10	No	3000	—	—	—	—	3000
III	10	No	4000	—	—	—	—	4000
IV	10	Yes	—	—	—	—	—	—
V	10	Yes	3000	—	—	—	—	3000
VI	10	Yes	4000	—	—	—	—	4000
VII	10	Yes	1200	1200	1200	1200	1200	6000
VIII	10	Yes	1600	1600	1600	1600	1600	8000

neck irradiated with single doses of 3000 rad. (*Falkmer et al* 1962 *Fors et al* 1964) *As a complement to the previous study* we have now investigated the effect of proton irradiation on *V×2 carcinoma in the anterior abdominal wall by single* and *fractionated treatment.*

Material and Methods

The experiments were performed on 80 female rabbits in 8 groups as shown in Table I

All experiments except those on Group II were performed simultaneously In the course of the investigation some animals became ill and died shortly afterwards The cause if this situation was probably an epidemic infection in the animal house which was difficult to combat No conclusions can therefore be drawn from observations concerning the survival weight changes and general condition of the animals, but some *information of interest can be obtained by comparing the results of the different* groups The original Group I was later supplemented by a new Group II these animals being treated in the same way as those *of Group I but with different housing conditions* The animals of Group II showed no signs of any infection

In the animals of Groups IV V VI VII and VIII V×2 carcinoma was implanted in the muscles of the anterior abdominal wall *medially* and immediately above the symphysis The rabbits

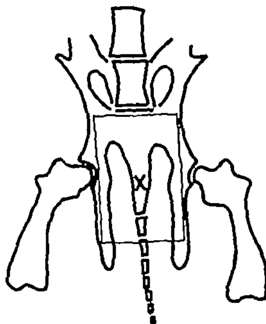


Fig. 1 Diagram based on roentgenogram showing the position of the radiation field, and the site of implantation of the V 2 carcinoma (X)

of Groups I II and III with no carcinoma, served as controls and for comparison with the results of previous investigations (see Fig. 3)

Implantation was performed ten days before irradiation. Tumour tissues for implantation were taken from a tumour extirpated immediately before operation and stored temporarily in physiological saline A grain-sized piece of tumour tissue was inserted through a small incision which was then sutured with catgut.

Ten days after implantation all wounds were practically healed, and without exception the tumour had grown to the size of a hazel nut. The individual weights of the rabbits (2.1–3.13 kg) varied little during the period between implantation and irradiation. All rabbits except those of Group IV were irradiated. The field was 4×6 cm^2 and located so that all pelvic organs were covered by the beam (see Fig. 1)

Table I *Plan of the Experiment (cf Fig 3).*

Group No	Number of Animals	Tumour Implanted	Dose in Rad of Radiation Seance No. 1	2	3	4	5	Total Dose (Rad)
I	10	No	3000	—	—		—	3000
II	10	No	3000	—	—	—	—	3000
III	10	No	4000	—	—	—	—	4000
IV	10	Yes	—	—	—	—	—	—
V	10	Yes	3000	—	—	—	—	3000
VI	10	Yes	4000	—	—	—	—	4000
VII	10	Yes	1200	1200	1200	1200	1200	6000
VIII	10	Yes	1600	1600	1600	1600	1600	8000

neck irradiated with single doses of 3000 rad. (*Falkmer et al* 1962 *Fors et al* 1964) As a complement to the previous study we have now investigated the effect of proton irradiation on V×2 carcinoma in the anterior abdominal wall by single and fractionated treatment.

Material and Methods

The experiments were performed on 80 female rabbits in 8 groups as shown in Table I

All experiments except those on Group II were performed simultaneously In the course of the investigation some animals became ill and died shortly afterwards The cause if this situa tion was probably an epidemic infection in the animal house which was difficult to combat No conclusions can therefore be drawn from observations concerning the survival, weight changes and general condition of the animals, but some information of interest can be obtained by comparing the results of the different groups. The original Group I was later supplemented by a new Group II, these animals being treated in the same way as those of Group I, but with different housing conditions The animals of Group II showed no signs of any infection

In the animals of Groups IV V VI VII and VIII V×2 carcinoma was implanted in the muscles of the anterior abdominal wall medially and immediately above the symphysis The rabbits

During the observation period following irradiation the animals were weighed twice a week. The site of implantation and the irradiated area were palpated and inspected once a week. Weber's test for blood in the faeces was performed twice a week. Haemoglobin and blood corpuscles were checked regularly each week in Groups I and VIII and occasionally in the other groups.

Results

Group I This group comprised 10 rabbits with no tumour and irradiated with single dose of 3000 rad. At the beginning of the experiment, the animals weighed 2.3–3.0 kg. During the second to sixth week after irradiation, epidermitis and epilation were observed in the irradiated area. From the sixth week the skin healed. No rabbit showed serious skin changes with ulceration. The haemoglobin concentration was 70–80 % and no significant changes were noted during the period of observation. The number of white blood corpuscles was 7 700–12 800 per mm³ Weber test was always negative. Immediately after irradiation one rabbit (No 686) showed a progressive weight fall and died after two weeks. The condition of the other nine animals was relatively normal until the fifth week after irradiation, when two rabbits (Nos. 685 and 687) showed deterioration W than 10.5 weeks these and five other animals had died, while two rabbits (Nos. 688 and 675) lived for 17 and 18 weeks respectively. The average weekly weight decrease was 75 g. (See Fig. 3)

At autopsy rabbit No. 686 which died in the second week after irradiation showed purulent pneumonia and partial necrosis of the lung. In three rabbits (Nos 675, 690 and 693) the proximal part of the rectum was dilated but without apparent microscopic changes in the rectal wall. With these exceptions, no macro- or microscopic radiation changes were found in this group in conformity with the observations made in the following group of healthy irradiated animals.

Group II This group comprised 10 rabbits with no tumour. The animals were irradiated with single dose of 3000 rad and were equivalent to Group I, except for the fact that there was no apparent incidental infection which could distort the results.

At the beginning of the experiment the animals weighed 1.8–2.6 kg. During the third to sixth week after irradiation, epilation and, in some cases, epidermitis were observed in the irradiated area. No ulceration was observed and all changes disappeared. The haemoglobin concentration was before the irradiation 65–80 % with an average of 73 % and the number of the white blood corpuscles was 8 500–22 400 with an average of 14 000 (5800 poly 8200 mono) per mm³. Some months after irradiation the haemoglobin concentration was found to be 70–90 % (average 78 %) and the white blood corpuscles were 10 000 (4400 poly 5600 mono) per mm³. The results of Weber test was normal.

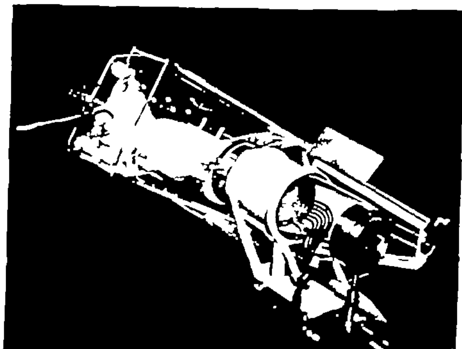


Fig. 2. The collimated 185 MeV proton beam passed through a 4×6 cm aperture, a parallel-plate ionization chamber a ridge filter (not seen in the picture) and a water absorber before it entered the water tank with the partly submersed animal. The water absorber was so adjusted that the plateau of the depth-dose curve contained the thickness of the animal. The roentgen film casset was removed before irradiation.

Before implantation, and also before irradiation, the rabbits were anaesthetized by Nembutal given intravenously. In five cases the intravenous injection was not successful and the anaesthetic was therefore given intraperitoneally with satisfactory results. No animals died during operation or irradiation.

In order to distribute the dose uniformly at all depths of the irradiated part of the body the animals were immersed in a water tank during irradiation (Fig 2) being fastened to a Lucite support and protected from direct contact with the water by a thin plastic bag. For all irradiations a "ridge filter" for a 5 cm length of the dose plateau was employed (*Larsson* 1961). Between the ridge filter and the animal a water absorber of suitable thickness was inserted so that the dose plateau contained the thickness of the animal. The dose rate in the plateau was 100–300 rad/min.

During the observation period following irradiation the animals were weighed twice a week. The site of implantation and the irradiated area were palpated and inspected once a week. Weber's test for blood in the faeces was performed twice a week. Haemoglobin and blood corpuscles were checked regularly each week in Groups I and VIII, and occasionally in the other groups.

Results

Group I This group comprised 10 rabbits with no tumour and irradiated with single dose of 3000 rad. At the beginning of the experiment, the animals weighed 2.3–3.0 kg. During the second to sixth week after irradiation, epidermitis and epilation were observed in the irradiated area. From the sixth week the skin healed. No rabbit showed serious skin changes with ulceration. The haemoglobin concentration was 70–80 % and no significant changes were noted during the period of observation. The number of white blood corpuscles was 7 700–12 800 per mm^3. Weber's test was always negative. Immediately after irradiation one rabbit (No 686) showed progressive weight fall and died after two weeks. The condition of the other nine animals was relatively normal until the fifth week after irradiation when two rabbits (Nos. 685 and 687) showed deterioration. Within 10.5 weeks these and five other animals had died, while two rabbits (Nos. 688 and 675) lived for 17 and 18 weeks, respectively. The average weekly weight decrease was 75 g. (See Fig. 3).

At autopsy rabbit No. 686 which died in the second week after irradiation showed purulent pneumonia and partial necrosis of the lung. In three rabbits (Nos. 673, 690 and 693) the proximal part of the rectum was dilated but without apparent microscopic changes in the rectal wall. With these exceptions no macro- or microscopic radiation changes were found in this group in conformity with the observations made in the following group of healthy irradiated animals.

Group II This group comprised 10 rabbits with no tumour. The animals were irradiated with a single dose of 3000 rad and were equivalent to Group I except for the fact that there was no apparent incidental infection which could distort the results.

At the beginning of the experiment the animals weighed 1.8–2.6 kg. During the third to sixth week after irradiation, epilation and, in some cases, epidermitis were observed in the irradiated area. No ulceration was observed and all changes disappeared. The haemoglobin concentration was before the irradiation 65–80 % with an average of 73 % and the number of the white blood corpuscles was 8 500–22 400 with an average of 14 000 (5800 poly 8200 mono) per mm^3. Some months after irradiation the haemoglobin concentration was found to be 70–90 % (average 78 %) and the white blood corpuscles were 10 000 (4400 poly 5600 mono) per mm^3. The results of Weber's test was normal.

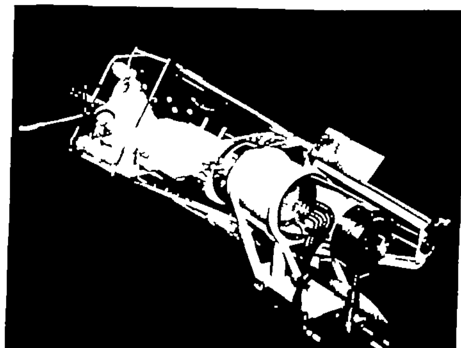


Fig 2. The collimated 185 MeV proton beam passed through a 4×6 cm² aperture a parallel-plate ionization chamber a ridge filter (not seen in the picture) and a water absorber before it entered the water tank with the partly submersed animal. The water absorber was so adjusted that the plateau of the depth-dose curve contained the thickness of the animal. The roentgen film casset was removed before irradiation.

Before implantation and also before irradiation the rabbits were anaesthetized by Nembutal given intravenously In five cases the intravenous injection was not successful and the ana esthetic was therefore given intraperitoneally with satisfactory results No animals died during operation or irradiation.

In order to distribute the dose uniformly at all depths of the irradiated part of the body the animals were immersed in a water tank during irradiation (Fig 2) being fastened to a Lucite support and protected from direct contact with the water by a thin plastic bag For all irradiations a ridge filter" for a 5 cm length of the dose plateau was employed (*Larsson* 1961) Between the ridge filter and the animal a water absorber of suitable thickness was inserted so that the dose plateau contained the thickness of the animal The dose rate in the plateau was 100–300 rad/min.

After irradiation the rabbits were active, ate normally and showed all signs of normal condition. The weight curves showed uniform increase, particularly during the first half year from 1.9 kg to 3.2 kg (average). One animal (No. 31) became slow and showed decrease in weight some months before it died 11 months after irradiation, of pneumonia and chronic inflammation of the liver and kidneys.

Two rabbits died within half year probably as result of vertebral fracture, one (No. 6) 1.5 months and the other (No. 24) 4 months after irradiation. Both animals were seemingly healthy until their last few days, but one morning showed paresis of the hind limbs and signs of bladder insufficiency.

During the second half year two animals were killed (Nos. 10 and 11) 9 months after irradiation. The animals had always been in good condition and showed a steady weight increase except for the last few weeks when they appeared less healthy with poor appetite and deteriorated general condition.

Three other rabbits (Nos 2, 3 and 19) were killed more than 1 year after irradiation. These rabbits were healthy and in a good general condition at the time of death with an average weight of 3.5 kg.

The two remaining rabbits were permitted to live longer. One (No. 13) had previously been apparently healthy and had doubled its weight, until few months before death when it suddenly became slow with anorrhexia and a poor general condition. It died 20 months after irradiation. The other rabbit (No. 14) was healthy and lively all the time and weighed 3.8 kg when killed 23 months after irradiation (See Fig. 3).

At the post-mortem examination of rabbit No. 31 which died 11 months after irradiation, both lungs were found to be partially atelectatic, permitting only poor ventilation. The lungs as well as the liver and the kidneys, showed large number of white and red spots. When studied in the microscope these spots showed pronounced fibrosis, necrosis and extensive calcification. The lungs were also pneumonic. The intestines were apparently normal.

At the autopsy of the three other rabbits which died spontaneously and of the six rabbits which were killed, no pathological findings were made macroscopically. On microscopic investigation, no radiation damage or other significant changes were observed.

Group III. This group comprised 10 rabbits without tumour and irradiated with a single dose of 4000 rad. At the start of the experiment the animals weighed 2.2–3.5 kg, with an average of 2.9 kg.

At the end of the second week after irradiation, epilation and dermatitis were observed in the irradiated area. The changes were most pronounced during the second to sixth weeks after irradiation, after which time healing occurred in all animals except for No. 661 in which the skin changes progressed until necrosis occurred centrally in the irradiated area. In five animals (Nos. 655, 656, 654, 610 and 661) the area around the anus and vulva was swollen.

The haemoglobin concentration was measured once in these animals, 3–4

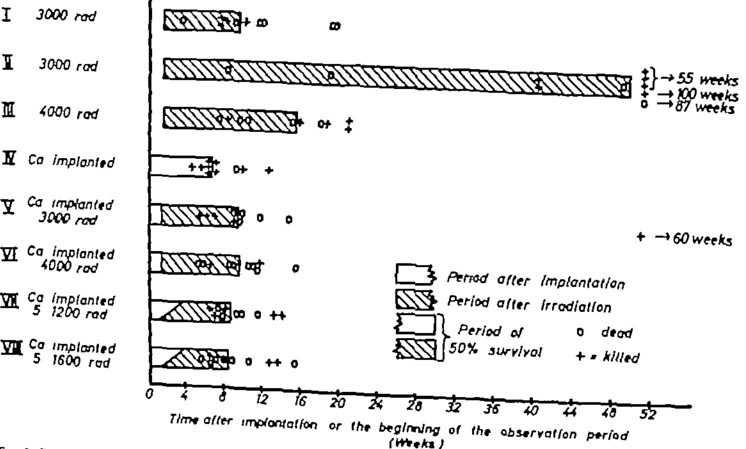


Fig. 3. Diagram demonstrating individual survival period in relation to the time of implantation and/or irradiation. The ends of the bars represent 50 % survival.

At cross-section, all tumours presented alternating solid and necrotic areas. Microscopically there was typical carcinoma in all cases.

Group V. This group comprised 10 rabbits with an implanted tumour. The animals were irradiated with a dose of 3000 rad. In all cases the tumour transplant showed continuous growth before irradiation, nine days after implantation, in eight animals to the size of a hazel nut, and in two animals (Nos. 633 and 624) to the size of a pea. The initial weight of these animals was between 2.6 and 3.4 kg.

The irradiated area presented an almost identical picture of skin changes. In the second week after irradiation, there was partial epilation particularly in the central part of the irradiated area, and also epidermitis mainly dry but also moist. No ulceration was observed. All skin changes progressed during the third to eight weeks after irradiation after which time clear regression was noted.

The implanted tumour decreased in size in the first two weeks after irradiation, and in eight of the animals it was then almost non-palpable. After 4–5 weeks abdominal metastases were palpable in seven animals and in three no tumours could be found (Nos. 624, 633 and 639). In this group the haemoglobin concentration was found to be 77.5 % and the number of white blood corpuscles 10,900 per mm^3 (average values). Weber test was negative in all cases except for one rabbit (No. 624) which showed a positive reaction on one occasion, one month after irradiation. The general condition of these animals was normal in spite of the weight decrease. Between the 5th and 10th weeks after irradiation there was marked change in the general condition and several animals became slow. At 10 weeks three rabbits had died spontaneously and four had been killed because of their poor condition, one of them after only five weeks. One rabbit (No. 624) which had appeared normal for 10 weeks, deteriorated suddenly and died one week later. One rabbit (No. 64.) died 15 weeks after the start of the experiment, of peritonitis caused by cancer-induced perforation. One rabbit (No. 639) was killed after 60 weeks because of fractured hind leg.

At the autopsy those rabbits (Nos. 624, 633 and 639) without palpable metastases and which died or were killed 9–60 weeks after irradiation, no carcinoma was found, either macroscopically or microscopically. In the other rabbits which died or were killed because of poor condition, 6 rabbits within 10 weeks and one after 15 weeks, cancer metastases were found in the abdominal cavity outside the irradiation area (Fig. 5). In five cases there were also cancer metastases in the lungs.

At the irradiated site of implantation no apparently viable cancer cells were demonstrated in any abbit (See Fig. 4). In one case, No. 623, there was an area of resistance 1 cm wide below the incision scar consisting of sclerotic connective tissue with some nests of necrotic cancer cells.

Group VI. This group comprised 10 rabbits with an implanted tumour. Irradiation was performed with single dose of 4000 rad. The initial weight was 2.3–3.6 kg (average 2.9 kg). The tumour was of the size of hazel nut at the time of irradiation. One to two weeks after irradiation there was

weeks after irradiation. On this occasion the values ranged between 67 and 89 % with an average of 81 % The number of white blood corpuscles per mm³ measured at the same time was 4900–13 400 with an average of 8700. Weber's test which was performed each week was in eight animals slightly positive on isolated occasions 2–4 months after irradiation and in one animal clearly positive on one occasion 4 months after irradiation. The faeces were solid except for two cases (Nos 655 and 659) in which diarrhoea was observed on single occasions.

Six weeks after irradiation deterioration of the general condition was noted and in nine weeks four rabbits died or had to be killed. These rabbits had a low initial weight (2.0–2.5 kg) The six remaining animals with initial weights of 3.0–3.5 kg lived considerably longer viz. 14–19 weeks (See Fig 3) One of these animals (No. 654) presented a genital prolapse 14.5 weeks after irradiation and was therefore killed. At the autopsy of this rabbit the uterus vagina and bladder were involved and the ureters and renal pelvis were dilated.

In one rabbit (No 610) which died 16 weeks after irradiation, at autopsy an old haematoma was found around the vagina, and a perforated rectum. There was a diffuse peritonitis. Similarly haematomas were found in two other rabbits (Nos 656 and 628) but no damage to the intestines was noted in these cases

The other rabbits of this group showed no macroscopic changes except for dilatation of the rectum. Microscopically no significant changes were observed.

Group IV This group comprised 10 rabbits with an implanted tumour No irradiation was performed In conformity with other animals with a transplanted tumour these rabbits presented a growth the size of a hazel nut at the site of implantation, about 10 days after the tumour was implanted. The tumour increased rapidly and was the size of an orange 1 month after implantation. After two months it showed a characteristic change of consistency from solid to soft and irregular In half of the rabbits Nos. 662, 650 644 635 and 645 perforation of the skin occurred through which necrotic tumour masses poured out. At the start of the experiment the animals weighed 2.2 3.3 kg with an average of 2 8 kg. The average weight decrease was 104 g per week. The survival period varied between 4.5 and 13 weeks with an average of 7 4 weeks. One rabbit died spontaneously (No 636) while the other animals were killed in a state of cachexia (See Fig 3) One month after the onset of the experiment the haemoglobin concentration was found to be 63–81 % (average 75 %) and the white blood corpuscles 20,200 (15 750 poly 4450 mono) per mm³ Weber's test performed each week, was found to be negative and no cases of diarrhoea were noted

The observations at autopsy were uniform and as follows. at the site of implantation in the anterior abdominal wall orange-sized tumours were found filling the lower abdominal cavity and also the pelvis Metastases were found in the lungs in six animals in the epicardium in one animal and in the liver in one animal.

At cross-section, all tumours presented alternating solid and necrotic areas. Microscopically there was a typical carcinoma in all cases.

Group V This group comprised 10 rabbits with an implanted tumour The animals were irradiated with dose of 3000 rad. In all cases the tumour transplant showed continuous growth before irradiation, nine days after implantation, in eight animals to the size of hazel nut, and in two animals (Nos. 633 and 624) to the size of a pea. The initial weight of these animals was between 2.6 and 3.4 kg.

The irradiated area presented an almost identical picture of skin changes. In the second week after irradiation, there was partial epilation, particularly in the central part of the irradiated area, and also epidermitis, mainly dry but also moist. No ulceration was observed. All skin changes progressed during the third to eight weeks after irradiation, after which time clear regression was noted.

The implanted tumour decreased in size in the first two weeks after irradiation, and in eight of the animals it was then almost non-palpable. After 4–5 weeks abdominal metastases were palpable in seven animals and in three no tumours could be found (Nos. 624 633 and 639) In this group the haemoglobin concentration was found to be 77.5 % and the number of white blood corpuscles 10,900 per mm^3 (average values) Weber test was negative in all cases except for one rabbit (No. 624) which showed a positive reaction on one occasion, one month after irradiation. The general condition of these animals was normal in spite of the weight decrease. Between the 5th and 10th weeks after irradiation there was marked change in the general condition and several animals became slow At 10 weeks three rabbits had died spontaneously and four had been killed because of their poor condition, one of them after only five weeks. One rabbit (No. 624) which had appeared normal for 10 weeks, deteriorated suddenly and died one week later. One rabbit (No 662) died 15 weeks after the start of the experiment of peritonitis caused by cancer-induced perforation. One rabbit (No. 639) was killed after 60 weeks because of fractured hind leg.

At the autopsy those rabbits (Nos 624 633 and 639) without palpable metastases and which died or were killed 9–60 weeks after irradiation, no carcinoma was found, either macroscopically or microscopically In the other rabbits which died or were killed because of poor condition, 6 rabbits, within 10 weeks and one after 15 weeks, cancer metastases were found in the abdominal cavity outside the irradiation area (Fig. 5) In five cases there were also cancer metastases in the lungs

At the irradiated site of implantation no apparently viable cancer cells were demonstrated in any rabbit (See Fig 4). In one case No. 623 there was an area of resistance 1 cm wide below the incision scar consisting of sclerotic connective tissue with some nests of necrotic cancer cells.

Group VI This group comprised 10 rabbits with an implanted tumour Irradiation was performed with a single dose of 4000 rad. The initial weight was 2.3–3.6 kg (average 2.9 kg) The tumour was of the size of hazel nut at the time of irradiation. One to two weeks after irradiation there was



Fig 4 Histologic section through the site of implantation of the V×2 carcinoma in the anterior abdominal wall. The animal was irradiated with 3000 rad 9 days after implantation and died 56 days after irradiation. No tumour cells could be seen in this site. Van Gieson ×275

slight decrease in its size and after one month it was no longer palpable with certainty As in the previous irradiated groups the skin changes were most pronounced 3–6 weeks after irradiation when epilation and epidermitis were observed within the central part of the irradiated area. In two cases (Nos. 612 and 594) severe changes occurred three weeks after irradiation with



Fig. 5. Histologic section showing metastasis of V×2 carcinoma in posterior part of the abdomen from the same animal as in fig. 4. Van Gieson ×275

dermal necrosis in small central region. In three cases (Nos. 632, 617 and 6.5) there was oedema around the anus and vulva. In six of the animals metastases were palpable in the abdominal cavity one month after irradiation (40 days after implantation).

The general condition of these animals and their weights were relatively unaffected until 3 weeks after irradiation. By the fifth to tenth weeks after irradiation five animals had deteriorated. Four of these died spontaneously



Fig 4 Histologic section through the site of implantation of the V×2 carcinoma in the anterior abdominal wall The animal was irradiated with 3000 rad 9 days after implantation and died 56 days after irradiation. No tumour cells could be seen in this site Van Gieson × 275

slight decrease in its size, and after one month it was no longer palpable with certainty As in the previous irradiated groups the skin changes were most pronounced 3–6 weeks after irradiation when epilation and epidermitis were observed within the central part of the irradiated area. In two cases (Nos. 612 and 594) severe changes occurred three weeks after irradiation with

(total dose 8000 rad) The initial weight was 2.4–3.2 kg (average 2.3 kg). The implanted tumour was of the size of hazel nut at the time of irradiation. In four rabbits (Nos 635 637 615 and 621) it had increased slightly by the 17th day after irradiation, but later diminished. At the end of the observation period the tumour was no longer palpable in three rabbits. In two rabbits (Nos 635 and 637) it was of the size of a pea. The skin changes were in conformity with those of previous groups and were most pronounced 2–6 weeks after irradiation. After this time the epidermitis and epilation regressed. In four cases however (Nos 625 649 621 and 643) necrosis was observed. In three rabbits there was oedema around the anus and vulva. In eight of the animals abdominal metastases were suspected 1 month after the start of the experiment

The general condition of the animals was unaffected until the 5th week after irradiation, when deterioration commenced. Between the 5th and 10th weeks six animals died spontaneously and one was killed because of its poor condition One animal (No 593) lived for 15 weeks and two (Nos. 619 and 621) for 13 weeks. The average survival period was 11.5 weeks. After eight weeks 50 % of the animals were dead (see Fig 3) The weight decreased from the time of the start of the experiment, more rapidly from the fifth week, and the final weight was between 1.7 and 2.8 kg (average 2.2 kg) The haemoglobin and white blood corpuscle values 1 month after irradiation were 76 % (59–85 %) and 10,500 (poly 6100 mono 4400) per mm³ respectively Weber' test, which was performed each week, was positive on single occasions in two animals (Nos 615 and 593)

At autopsy no cancer was found in two rabbits (Nos. 621 and 593) which died 13 and 15 weeks after the start of the experiment In the remaining eight rabbits cancer metastases were found in the abdominal cavity and in seven of these cases also in the lungs

In two rabbits with palpable growths in the anterior abdominal wall one (No 635) presented tumour the size of hazel nut infiltrating the abdominal organs, and the other (No. 637) pea-sized tumour at the site of implantation On microscopic examination of the site of implantation, only necrotic cancer cells were found in these animals.

At autopsy an old perivaginal haematoma was found in one animal (No. 693) and a dilated rectum in another (No. 615)

Discussion

The tumour-bearing and proton-irradiated rabbits of groups V VI, VII and VIII died shortly after irradiation only 15 of 40 animals h ed after 10 weeks. In seven of these, no carcinoma was found, nor in two rabbits which died after 9 5 weeks. The remaining 31 rabbits had external e metastases at the time of the autopsy indicating that the carcinoma had spread rapidly to the extent

and one was killed. One rabbit, No. 625 lived for 15.5 weeks and four rabbits for 11–13 weeks (see Fig. 3) The haemoglobin concentration one month after irradiation was 75 % and the average number of white blood corpuscles 10 100 (4700 poly 5400 mono) per mm^3 Weber's test performed each week was negative No cases of diarrhoea were noted The average survival period was 8 weeks in the animals in which cancer metastases were observed. The other animals survived for an average of 12.4 weeks

At autopsy no cancer was demonstrated in three rabbits (Nos. 641 617 and 625) which died 10 11 and 15.5 weeks after the start of the experiment. In the other rabbits cancer metastases were found in the abdominal cavity in the upper part of the posterior wall In addition, three of the latter animals showed metastases in the lungs.

At the irradiated site of implantation, no cancer was found at autopsy either macroscopically or microscopically

At autopsy a perivaginal haematoma was found in one case (No. 625) In two rabbits (Nos. 694 and 641) there were intestinal changes, in the first case some constriction of the colon, and in the second case a dilated rectum. In the latter case microscopic examination revealed moderate cell infiltration in the rectal wall.

Group VII This group comprised 10 rabbits with an implanted tumour All animals showed a tumour the size of a hazel nut 10 days after implantation, when irradiation was performed. The initial weight was 1 4–3.2 kg

The animals were irradiated with a dose of 1200 rad on five consecutive days thus with a total dose of 6000 rad Within 2–3 weeks after irradiation epilation and moist epidermitis were noted. Swelling after and oedema around the anus and vulva were observed in six of the animals after the third week. After about five weeks the skin changes regressed except in one rabbit (No. 595) in which the progressive changes led to central necrosis. The implanted tumour regressed in all cases and was not palpable after one month Metastases were palpable in the abdominal cavity in six cases about five weeks after the start of the irradiation. The haemoglobin concentration and white blood corpuscles showed normal values (75 % and 9900 mm^3 respectively) Weber's test was negative in all cases. Weight decrease was noted in all cases

Between the 7th and 10th week, four animals died spontaneously and three were killed because of their poor condition The three remaining animals (Nos 595 606 and 620) lived for 11–13 weeks (see Fig. 3)

In one animal (No. 611) which died 2 months after the treatment, no cancer was seen at autopsy In the other cases cancer was present one rabbit (No 620) showed lung metastases alone and the others three of which also had lung metastases, showed cancer in the abdominal cavity

One rabbit (No 595) showed viable cancer at the site of implantation in the irradiated region which furthermore was sclerotic. In the other rabbit no carcinoma was observed at th site of implantation.

Group VIII This group comprised 10 animals with an implanted tumour The animals were irradiated with a dose of 1600 rad on five consecutive days

signs of radiation damage were noted. These results indicate that if incidental diseases can be avoided the rabbit tolerates a single 3000 rad dose in the pelvis. From previously published experiments on radiation effects on V×2 carcinoma in the rabbit uterus it appears that treatment with proton radiation in a dose of 3000 or 4000 rad leads to regression of the carcinoma. The results of the present investigation, which covers a considerably larger material support this view. Thus the experiments of Group V showed that in no case did carcinoma implanted in the anterior abdominal wall survive single irradiation with 3000 rad. Irradiation with 4000 rad in Group VI gave the same result.

The main aim of the present study was to ascertain whether fractionated irradiation gave advantages over the single irradiation procedure. To this end Group VII was treated with 5×1200 rad, and Group VIII with 5×1600 rad. The former radiation schedule should correspond to a single treatment of 3000 rad if Strandqvist's diagram can be considered valid for the situation considered here. It is clear from the results that the effect of irradiation with 5×1200 rad for 5 days on the implanted carcinoma was in fact approximately similar to but not better than that of single treatment with 3000 rad. The two schemes might therefore be considered equivalent from this point of view, an opinion that is not invalidated by the fact that viable carcinoma was found in one rabbit treated by fractionated irradiation but not after single treatment with 3000 rad. After fractionated treatment with 5×1600 rad for five days (Group VIII) the carcinoma appeared to have disappeared from the site of implantation in all animals. This result is in conformity with the corresponding observations after treatment of the animals of Group VI with a single dose of 4000 rad.

It is of interest to compare these results with our earlier observations in a case of metastatizing mammary carcinoma in a 58 year old woman (*Falkmer et al.* 1962). This patient was irradiated for skin metastases: proton irradiation had been performed on one tumour with a single dose of 3000 rad and on four tumours by fractionated irradiation with varying doses, three times in seven days. After single irradiation the tumour showed apparently complete regression in 3 weeks, and 9 weeks later no cancer was

that it might have contributed to or caused the death of the animal The length of the time which the V×2 carcinoma needs to kill a rabbit after implantation is indicated by the results of Group IV in which no radiation was applied These rabbits all died within 12 weeks nine of them within 9 weeks.

With the exception of the animals of Group II which were apparently free from any epidemic animal house infection and mostly lived for more than fourty weeks (Fig 3) the animals without an implanted tumour died relatively early but not in so short a time as the above mentioned rabbits with implanted carcinoma. Of 20 rabbits in Groups I and III irradiated with single doses of 3000 or 4000 rad, 12 died within 12 weeks while the remaining 8 animals died between 14 and 20 weeks after irradiation. It may be noted that in Group III, irradiated with 4000 rad, the heavier animals generally showed longer survival. Thus five rabbits with initial weights of 3 0–3 5 kg survived for 14–19 weeks while 3 out of 4 rabbits weighing 2.0–2.5 kg died as early as 6–7.5 weeks after irradiation As expected, 4000 rad caused more severe damage than 3000 rad This was accentuated by the fact that one animal in Group III showed skin necrosis and that six animals of the same group showed oedema and swelling around the anus and vulva. Furthermore in three animals of Group III hematomas were found around genital tract at autopsy and in one case perforation of rectum with peritonitis was observed. No such damage was seen in the animals of groups I and II irradiated with 3000 rad.

The rabbit seemed to tolerate single irradiation with 3000 rad relatively well, as indicated by the mild reaction shown by the animals of Group II which were treated under better housing conditions The fact that their average weight, half a year after irradiation, was nearly 40 per cent higher than at the start of the experiment lends support to this opinion. These animals were kept under optimal conditions after irradiation and showed no signs of deterioration. Half the animals lived for more than one year Four animals died spontaneously two of them probably as a result of a fracture of the vertebral column. Six animals were killed at various times up to 13 months after irradiation No rabbit appeared to have died as a consequence of the irradiation, and no

signs of radiation damage were noted. These results indicate that if incidental diseases can be avoided the rabbit tolerates a single 3000 rad dose in the pelvis. From previously published experiments on radiation effects on V×2 carcinoma in the rabbit uterus it appears that treatment with proton radiation in a dose of 3000 or 4000 rad leads to regression of the carcinoma. The results of the present investigation, which covers a considerably larger material support this view Thus the experiments of Group V showed that in no case did carcinoma implanted in the anterior abdominal wall survive single irradiation with 3000 rad. Irradiation with 4000 rad in Group VI gave the same result.

The main aim of the present study was to ascertain whether fractionated irradiation gave advantages over the single irradiation procedure. To this end Group VII was treated with 5×1200 rad, and Group VIII with 5×1600 rad. The former radiation schedule should correspond to a single treatment of 3000 rad if Strandqvist's diagram can be considered valid for the situation considered here. It is clear from the results that the effect of irradiation with 5×1200 rad for 5 days on the implanted carcinoma was in fact approximately similar to but not better than that of single treatment with 3000 rad. The two schemes might therefore be considered equivalent from this point of view an opinion that is not invalidated by the fact that viable carcinoma was found in one rabbit treated by fractionated irradiation but not after single treatment with 3000 rad. After fractionated treatment with 5×1600 rad for five days (Group VIII) the carcinoma appeared to have disappeared from the site of implantation in all animals. This result is in conformity with the corresponding observations after treatment of the animals of Group VI with a single dose of 4000 rad

It is of interest to compare these results with our earlier observations in a case of metastatizing mammary carcinoma in a 58 year old woman (*Falkmer et al.* 1962) This patient was irradiated for skin metastases proton irradiation had been performed on one tumour with a single dose of 3000 rad and on four tumours by fractionated irradiation with varying doses, three times in seven days. After single irradiation the tumour showed apparently complete regression in 3 weeks, and 9 weeks later no cancer was

found in biopsy material Four tumours treated by fractionated irradiation with 4500 6000 7000 and 7500 rad, respectively showed varying degrees of regression It was found that 4500 rad did not succeed in causing complete regression of the carcinoma. With 6000 rad partly degenerated cancer cells could still be obtained on the 31st day After irradiation with 7000 rad and 7500 rad no cancer was found at biopsy These observations indicate that a single irradiation with 3000 rad causes changes of the cancer cells equivalent to those found after fractionated treatment with approximately 6000 rad in one week. This result conforms well with those from the present experimental study in the rabbit.

Concerning the effect of radiation on healthy tissues fractionated irradiation with 6000 rad in Group VII caused skin necrosis in one case and oedema and reddening around the anus and vulva in 6 cases No such damage was found in the rabbits of Groups I II and V which were given single irradiation with 3000 rad. These observations also conform well with the findings concerning skin damage in the patient mentioned above After fractionated irradiation with 6000 rad, necrosis was observed in the skin, while no such damage was observed after a single treatment with 3000 rad

It is thus clear that single irradiation with 3000 rad in the rabbit causes regression of V×2 carcinoma without causing any serious damage to the healthy rabbit tissues and that fractionated treat ment with 6000 rad does not seem to offer any advantage under the experimental conditions employed.

SUMMARY

The first part of this study concerns the effect of single proton irradiation with 3000 and 4000 rad on healthy rabbits and on rabbits with V×2 carcinoma implanted in the lower abdominal wall. With the heavier dose and particularly in the smaller animals, damage was observed in the skin and also in the internal organs. With 3000 rad no such damage was observed. The results also verify previous observations that a single radiation with 3000 rad causes regression of V×2 carcinoma

In the second part of the study fractionated treatment was

applied. The results indicate that a dose of 1200 rad given on 5 consecutive days does not give better therapeutic results than single irradiation with 3000 rad under the experimental conditions applied. If there is any difference between the results of the different irradiation schemes, it is probably in favour of the single radiation. This latter conclusion is based on the observation that erythema and oedema around the anus and vulva and also necrosis of the skin were observed among animals treated by fractionated irradiation but not among those given a single dose which was equivalent in its tumour-killing capacity.

Acknowledgement

This work has been supported by grants from the Swedish cancer society and from the Swedish medical research council.

We are also indebted to professor Sture Eriksson, Ultuna for placing facilities for animal housing at our disposal.

REFERENCES

Falkmer S, Larsson, B and *Stenson S.* Acta Radiol. 52 217 1959
Falkmer S. Fors, B Larsson B Lindell A. Naeslund, J and *Stenson S.* Acta Radiol. 58 33 1962
Fors B Larsson, B Lindell, A. Naeslund J and *Stenson S* Acta Radiol. 2 384 1964
Larsson, B Brit J Radiol 34 143 1961
Naeslund, J Stenson, S Falkmer S Lindell A. Larsson B and *Sandberg, T* Acta Soc Med Upsal 63 155 1958
Naeslund, J Stenson S and *Larsson, B* Acta Obstet. Gynec. Scand. 38 563, 1959

Received on Sept 1 1967

found in biopsy material Four tumours treated by fractionated irradiation with 4500 6000 7000, and 7500 rad, respectively showed varying degrees of regression. It was found that 4500 rad did not succeed in causing complete regression of the carcinoma. With 6000 rad partly degenerated cancer cells could still be obtained on the 31st day After irradiation with 7000 rad and 7500 rad no cancer was found at biopsy These observations indicate that a single irradiation with 3000 rad causes changes of the cancer cells equivalent to those found after fractionated treatment with approximately 6000 rad in one week. This result conforms well with those from the present experimental study in the rabbit

Concerning the effect of radiation on healthy tissues, fractionated irradiation with 6000 rad in Group VII caused skin necrosis in one case and oedema and reddening around the anus and vulva in 6 cases No such damage was found in the rabbits of Groups I, II and V which were given single irradiation with 3000 rad. These observations also conform well with the findings concerning skin damage in the patient mentioned above After fractionated irradiation with 6000 rad necrosis was observed in the skin, while no such damage was observed after a single treatment with 3000 rad.

It is thus clear that single irradiation with 3000 rad in the rabbit causes regression of V×2 carcinoma without causing any serious damage to the healthy rabbit tissues and that fractionated treatment with 6000 rad does not seem to offer any advantage under the experimental conditions employed.

SUMMARY

The first part of this study concerns the effect of single proton irradiation with 3000 and 4000 rad on healthy rabbits and on rabbits with V×2 carcinoma implanted in the lower abdominal wall. With the heavier dose and particularly in the smaller animals, damage was observed in the skin and also in the internal organs. With 3000 rad no such damage was observed. The results also verify previous observations that a single radiation with 3000 rad causes regression of V×2 carcinoma

In the second part of the study fractionated treatment was

applied. The results indicate that a dose of 1200 rad given on 5 consecutive days does not give better therapeutic results than single irradiation with 3000 rad under the experimental conditions applied. If there is any difference between the results of the different irradiation schemes, it is probably in favour of the single radiation. This latter conclusion is based on the observation that erythema and oedema around the anus and vulva and also necrosis of the skin were observed among animals treated by fractionated irradiation but not among those given a single dose which was equivalent in its tumour-killing capacity

Acknowledgement

This work has been supported by grants from the Swedish cancer society and from the Swedish medical research council.

We are also indebted to professor Sture Eriksson, Ultuna for placing facilities for animal housing at our disposal.

REFERENCES

Falkmer S Larsson B. and Stenson, S Acta Radiol. 52 217 1959
Falkmer S Fors, B Larsson B Lindell A. Naeslund J and *Stenson, S.* Acta Radiol 58 33, 1962
Fors B Larsson B Lindell, A. Naeslund, J and Stenson S Acta Radiol. 2 384 1964
Larsson B Brit J Radiol 34 143 1961
Naeslund, J Stenson S *Falkmer S. Lindell, A. Larsson, B.* and *Svedberg, T* Acta Soc Med Upsal 63 155 1958
Naeslund, J Stenson S and Larsson, B. Acta Obstet. Gynec. Scand. 38 563 1959

Received on Sept 1 1967

Törnqvist, Åke Acta obst et gynec scandinav 47 391, 1968
From the Department of Obstetrics and Gynaecology (Professor S. Brody)
University of Goteborg, Sweden

THE EFFECTS OF EXOGENOUS HISTAMINE ON THE FOREARM BLOOD FLOW IN PREGNANT AND NON PREGNANT WOMEN BEFORE AND AFTER INHIBITION OF HISTAMINASE

BY

ÅKE TÖRNQVIST

In a previous investigation (*Törnqvist* 1968) the effects of infused histamine on the pulse rate was studied in pregnant and non-pregnant women before and after inhibition of histaminase by means of aminoguanidine Infusion of histamine increased the pulse rate in all subjects but the increase was more pronounced in the non-pregnant cases. After inhibition of histaminase the increase in pulse rate was about equal in the pregnant and the non-pregnant groups during infusion of histamine. The conclusion was drawn that the increased plasma histaminase activity during human pregnancy might possibly modify the sensitivity to exogenous histamine

Histamine is also a potent peripheral vasodilatator in animals (*Burn* and *Dale* 1926 *Folkow Haeger* and *Kahlson* 1948 *Kjellmer* and *Odelram* 1964) and in man (*Harmer* and *Harris* 1926 *Weiss Robb* and *Ellis* 1932 *Wakim et al.* 1949 *Duff et al* 1953 and 1954 *Fox et al.* 1961) *Wakim et al.* (1949) demonstrated that histamine infused intravenously increased the blood flow in proportion to the dosage, especially in the upper extremities *Duff et al* (1953 and 1954) and *Fox et al* (1961) showed an increase in forearm blood flow in man during intra-arterial infusion of histamine *Fox et al.* (1961) also showed an increase in blood flow during intravenous infusion of histamine but the

per 100 ml) The histamine was infused in periods which were always 6 minutes long. The interval between successive infusions of histamine was always 30 minutes. During the histamine-free periods physiological saline solution was infused at a low speed to prevent clotting in the needle and the vein.

Measurement of the forearm blood flow and calculation of the peripheral vascular resistance The forearm blood flow was measured by venous occlusion plethysmography For general principles see *Barcroft* and *Swan* (1953) In the plethysmograph the forearm was surrounded by a rubber sleeve of thin latex rubber Various sizes of sleeve were available to fit any subject. The ends of the sleeves were fastened to diaphragms of soft rubber three mm thick. The circulation through the hand could be interrupted with a 6 cm wide sphygmomanometer cuff on the wrist. An ordinary sphygmomanometer cuff 12 cm wide was applied proximal to the plethysmograph. During the recordings the temperature in the plethysmograph was held constant at 34 C. The temperature in the room was thermostatically maintained at 22 C For blood flow determinations the pressure in the cuffs was simultaneously increased to 40 mm Hg Blood flow determinations were made every 30 seconds during the infusions of histamine. The first blood flow determination was made about 30 seconds after the beginning of the infusion. Blood flow determinations at the same intervals, were also made for at least two minutes immediately before every infusion of histamine. All recordings were made in the right forearm and the blood flow was expressed as ml per minute and per 100 ml of tissue. Simultaneous recordings of the arterial blood pressure were made in the left arm by the conventional sphygmomanometer cuff method and auscultation of Korotkov s sound. The peripheral vascular resistance to blood flow in the forearm was expressed in peripheral resistance units (PRU) (*Green Lewis Nickerson* and *Heller* 1944) Functional mean arterial blood pressure was taken as the diastolic pressure plus 1/3 of the pulse pressure All patients were kept comfortable in bed for 30 minutes after the plethysmograph and the infusion needle were fitted before any recordings were made

increase was not so pronounced. *Kjellmer* and *Odelram* (1964) studied the effect of intra-arterial infusions of histamine on isolated preparations of cats calf muscle There was a decrease in vascular resistance to blood flow *Harmer* and *Harris* (1926) were unable to show any apparent increase in blood flow to the limbs as a whole even if they demonstrated an increase in the blood flow of the skin after injection of histamine. In their investigations they used a technique of single subcutaneous and intravenous injections of histamine

There are so far no reports on the effect of infused histamine on the forearm blood flow and on the peripheral vascular resistance in pregnant women In the present investigation these parameters were chosen to study the effect of infused histamine in pregnant and non-pregnant women If the increased plasma histaminase activity during human pregnancy modifes the effect of exogenous histamine inhibition of histaminase might possibly change the response to histamine in pregnant women

Material

Twenty-one pregnant and thirteen non pregnant women were investigated. The pregnant women were admitted to the clinic for legal abortion Their ages ranged from 18 to 42 years with a mean of 29 years Their body weights varied from 45 to 92 kg with a mean of 66 kg They were in the 16th to the 20th week of pregnancy counting from the first day of the last menstrual period The thirteen non pregnant women had all normal menstrual periods Most of them belonged to the nursing staff Their ages ranged from 20 to 45 years with a mean of 30 years Their body weights varied from 49 to 60 kg with a mean of 57 kg

Methods

Infusion of histamine Histamine was infused at a constant rate through an indwelling needle in the long saphenous vein at the ankle by means of a motor-driven syringe The dose rate was always 12 micrograms of histamine per minute (Histamine dihydrochloride, E. Merck A. G) The histamine was dissolved in physiological saline solution (1 mg of histamine dihydrochloride

Table I. *Histamine Infusion in Non-Pregnant Women*

Case	Increase in Forearm Blood Flow before Inhibition of Histaminase	Decrease in Peripheral Vascular Resistance before Inhibition of Histaminase	Increase in Forearm Blood Flow after Inhibition of Histaminase	Decrease in Peripheral Vascular Resistance after Inhibition of Histaminase
1	2.67 (2.34 ± 0.31)	17.4 (34.6)	2.03 (3.85 ± 0.22)	6.6 (20.8)
2	1.09 (2.96 ± 0.34)	7.4 (31.3)	1.64 (2.76 ± 0.17)	11.0 (33.3)
3	1.12 (1.87 ± 0.26)	18.8 (50.6)	1.19 (2.54 ± 0.26)	10.2 (37.0)
4	0.76 (5.12 ± 1.12)	1.8 (16.7)	1.28 (5.12 ± 0.86)	2.9 (17.3)
5	1.89 (4.03 ± 0.38)	7.6 (21.7)	0.27 (4.58 ± 0.67)	2.4 (21.1)
6	0.81 (1.78 ± 0.16)	14.4 (46.3)	0.44 (2.28 ± 0.38)	6.9 (38.1)
7	0.93 (3.30 ± 0.69)	4.7 (26.2)	1.50 (2.31 ± 0.26)	11.4 (36.4)
8	4.12 (2.42 ± 0.73)	26.7 (41.2)	4.07 (3.33 ± 1.18)	15.3 (27.3)
9	0.64 (3.33 ± 0.34)	0.9 (24.8)	0.27 (2.94 ± 0.35)	3.6 (29.8)
10	3.77 (3.96 ± 0.41)	9.7 (22.9)	1.91 (4.61 ± 0.63)	5.1 (20.5)
11	0.50 (1.98 ± 0.19)	8.1 (51.6)	0.24 (2.13 ± 0.41)	5.8 (48.7)
12	1.77 (2.74 ± 0.27)	11.6 (30.7)	1.59 (3.41 ± 0.18)	8.2 (24.4)
13	1.91 (3.22 ± 0.17)	10.5 (30.3)	1.55 (4.38 ± 0.71)	4.9 (21.5)
Mean	1.69	10.7	1.38	7.3

Infusion rate of histamine 12 micrograms per minute.
Forearm blood flow in ml/min × 100 ml ± standard deviation
Peripheral vascular resistance in peripheral resistance units (PRU)
Figures in brackets indicate resting forearm blood flow and resting peripheral vascular resistance before infusion of histamine.

respects with one testing method the tests were not independent and a significance level of 2.5 per cent was chosen, in each test to ensure that the significance level of the combined tests was at least, on the 5 per cent level.

Results

Table I shows the effect of the infused histamine on the forearm blood flow and on the peripheral vascular resistance in the non pregnant control group before and after inhibition of histaminase. In all cases histamine gave an increase in the blood flow and a decrease in the peripheral vascular resistance to blood flow. The effect of the histamine was not increased after inhibition of hista

Inhibition of histaminase Inhibition of histaminase was achieved by the intramuscular injection of 0 2 mg of aminoguanidine sul phate (Eastman Organical Chemicals) per kg of body weight The injected solution contained 10 mg of aminoguanidine sul phate per ml of sterilised water In a study of the effect of intra muscular injections of aminoguanidine sulphate on the plasma histaminase activity in pregnant women 30 minutes proved suf ficient for complete inhibition of the enzyme histaminase in blood plasma (*Törnqvist* unpublished)

Calculation of the changes in forearm blood flow and in peri pheral vascular resistance during infusion of histamine The dif ference between an average value of all blood flow recordings during infusion of histamine and an average value of 5 successive resting blood flow values immediately before the infusion was calculated and taken as a measure of the effect of the infused histamine on the blood flow in the forearm. The difference in peripheral vascular resistance was calculated in a similar way Immediately after the end of the histamine infusion aminogua nidine was injected intramuscularly Thirty minutes later hista mine was infused as before and the effects on the blood flow and on the peripheral vascular resistance were calculated. In 8 pregnant women histamine was infused on two occasions 30 minutes apart, without interposed inhibition of histaminase The injection of aminoguanidine was in these cases replaced by an intramuscular injection of physiological saline solution The in vestigation of these 8 women was done to investigate the response to exogenous histamine at repeated infusions.

Statistics

The response of the pregnant and the non pregnant groups to histamine, before inhibition of histaminase were compared. The change in the effects of the histamine caused by inhibition of histaminase in the pregnant and the non pregnant groups was also compared. The significance of differences between groups was tested by means of the Wilcoxon two-sample rank test (*Brownlee* 1961) As the same results were analyzed in two

Table I. *Histamine Infusion in Non-Pregnant Women*

Case	Increase in Forearm Blood Flow before Inhibition of Histaminase	Decrease in Peripheral Vascular Resistance before Inhibition of Histaminase	Increase in Forearm Blood Flow after Inhibition of Histaminase	Decrease in Peripheral Vascular Resistance after Inhibition of Histaminase
1	2.67 (2.34 ± 0.31)	17.4 (34.6)	2.03 (3.85 ± 0.22)	6.6 (20.8)
2	1.09 (2.96 ± 0.34)	7.4 (31.3)	1.64 (2.76 ± 0.17)	11.0 (33.3)
3	1.12 (1.87 ± 0.26)	18.8 (50.6)	1.19 (2.54 ± 0.26)	10.2 (37.0)
4	0.76 (5.12 ± 1.12)	1.8 (16.7)	1.28 (5.12 ± 0.86)	2.9 (17.3)
5	1.89 (4.03 ± 0.38)	7.6 (21.7)	0.77 (4.58 ± 0.67)	2.4 (21.1)
6	0.81 (1.78 ± 0.16)	14.4 (48.3)	0.44 (2.28 ± 0.36)	6.9 (38.1)
7	0.53 (3.30 ± 0.09)	4.7 (26.2)	1.50 (2.31 ± 0.26)	11.4 (36.4)
8	4.12 (2.42 ± 0.73)	26.7 (41.2)	4.07 (3.33 ± 1.18)	15.3 (27.3)
9	0.64 (3.33 ± 0.34)	0.9 (24.8)	0.27 (2.94 ± 0.35)	3.6 (29.8)
10	3.77 (3.96 ± 0.41)	9.7 (22.9)	1.91 (4.61 ± 0.63)	5.1 (20.5)
11	0.50 (1.98 ± 0.19)	8.1 (51.6)	0.24 (2.13 ± 0.41)	5.8 (48.7)
12	1.77 (2.74 ± 0.27)	11.6 (30.7)	1.59 (3.41 ± 0.18)	8.2 (24.4)
13	1.91 (3.22 ± 0.17)	10.5 (30.3)	1.55 (4.38 ± 0.71)	4.9 (21.5)
Mean	1.69	10.7	1.38	7.3

Infusion rate of histamine 12 micrograms per minute.
Forearm blood flow in ml/min × 100 ml ± standard deviation.
Peripheral vascular resistance in peripheral resistance units (PRU)
Figures in brackets indicate resting forearm blood flow and resting peripheral vascular resistance before infusion of histamine

respects with one testing method the tests were not independent and a significance level of 2.5 per cent was chosen, in each test, to ensure that the significance level of the combined tests was, at least on the 5 per cent level.

Results

Table I shows the effect of the infused histamine on the forearm blood flow and on the peripheral vascular resistance in the non-pregnant control group before and after inhibition of histaminase. In all cases histamine gave an increase in the blood flow and a decrease in the peripheral vascular resistance to blood flow The effect of the histamine was not increased after inhibition of hista

Inhibition of histaminase Inhibition of histaminase was achieved by the intramuscular injection of 0 2 mg of aminoguanidine sul phate (Eastman Organical Chemicals) per kg of body weight. The injected solution contained 10 mg of aminoguanidine sul phate per ml of sterilised water In a study of the effect of intra muscular injections of aminoguanidine sulphate on the plasma histaminase activity in pregnant women 30 minutes proved suf ficient for complete inhibition of the enzyme histaminase in blood plasma (*Törnqvist unpublished*)

Calculation of the changes in forearm blood flow and in peri pheral vascular resistance during infusion of histamine The dif ference between an average value of all blood flow recordings during infusion of histamine and an average value of 5 successive resting blood flow values immediately before the infusion was calculated and taken as a measure of the effect of the infused histamine on the blood flow in the forearm. The difference in peripheral vascular resistance was calculated in a similar way Immediately after the end of the histamine infusion aminogua nidine was injected intramuscularly Thirty minutes later hista mine was infused as before and the effects on the blood flow and on the peripheral vascular resistance were calculated. In 8 pregnant women histamine was infused on two occasions 30 minutes apart without interposed inhibition of histaminase The injection of aminoguanidine was in these cases replaced by an intramuscular injection of physiological saline solution The in vestigation of these 8 women was done to investigate the response to exogenous histamine at repeated infusions

Statistics

The response of the pregnant and the non-pregnant groups to histamine before inhibition of histaminase were compared. The change in the effects of the histamine caused by inhibition of histaminase in the pregnant and the non pregnant groups was also compared. The significance of differences between groups was tested by means of the Wilcoxon two-sample rank test (*Brownlee* 1961) As the same results were analysed in two

Table I. *Histamine Infusion in Non-Pregnant Women*

Case	Increase in Forearm Blood Flow before Inhibition of Histaminase	Decrease in Peripheral Vascular Resistance before Inhibition of Histaminase	Increase in Forearm Blood Flow after Inhibition of Histaminase	Decrease in Peripheral Vascular Resistance after Inhibition of Histaminase
1	2.67 (2.34 ± 0.31)	17.4 (34.6)	2.03 (3.85 ± 0.22)	6.6 (20.8)
2	1.09 (2.96 ± 0.34)	7.4 (31.3)	1.64 (2.76 ± 0.17)	11.0 (33.3)
3	1.12 (1.87 ± 0.26)	18.8 (50.6)	1.19 (2.54 ± 0.26)	10.2 (37.0)
4	0.76 (5.12 ± 1.12)	1.8 (16.7)	1.28 (5.12 ± 0.86)	2.9 (17.3)
5	1.89 (4.03 ± 0.38)	7.6 (21.7)	0.27 (4.58 ± 0.67)	2.4 (21.1)
6	0.81 (1.78 ± 0.16)	14.4 (46.3)	0.44 (2.28 ± 0.38)	6.9 (38.1)
7	0.93 (3.30 ± 0.69)	4.7 (26.2)	1.50 (2.31 ± 0.26)	11.4 (36.4)
8	4.12 (2.42 ± 0.73)	26.7 (41.2)	4.07 (3.33 ± 1.18)	15.3 (27.3)
9	0.64 (3.33 ± 0.34)	0.9 (24.8)	0.27 (2.94 ± 0.36)	3.6 (29.8)
10	3.77 (3.96 ± 0.41)	9.7 (22.9)	1.91 (4.61 ± 0.63)	5.1 (20.5)
11	0.50 (1.98 ± 0.19)	8.1 (51.6)	0.24 (2.13 ± 0.41)	5.8 (48.7)
12	1.77 (2.74 ± 0.27)	11.6 (30.7)	1.59 (3.41 ± 0.18)	8.2 (24.4)
13	1.91 (3.22 ± 0.17)	10.5 (30.3)	1.53 (4.38 ± 0.71)	4.9 (21.5)
Mean	1.69	10.7	1.38	7.3

Infusion rate of histamine 12 micrograms per minute.
Forearm blood flow in ml/min × 100 ml. ± standard deviation
Peripheral vascular resistance in peripheral resistance units (PRU)
Figures in brackets indicate resting forearm blood flow and resting peripheral vascular resistance before infusion of histamine.

respects with one testing method, the tests were not independent and a significance level of 2.5 per cent was chosen in each test, to ensure that the significance level of the combined tests was at least, on the 5 per cent level.

Results

Table I shows the effect of the infused histamine on the forearm blood flow and on the peripheral vascular resistance in the non-pregnant control group before and after inhibition of histaminase. In all cases histamine gave an increase in the blood flow and a decrease in the peripheral vascular resistance to blood flow. The effect of the histamine was not increased after inhibition of hista-

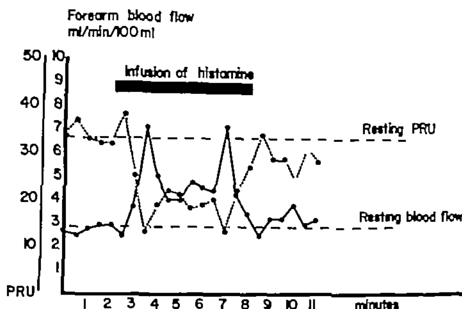


Fig 1 Graphical representation of the effect of an intravenous infusion of histamine on the forearm blood flow and on the peripheral vascular resistance in one non-pregnant woman. The enzyme histaminase was not inhibited. The blood flow (solide line) in ml per minute and per 100 ml of tissue. The vascular resistance (dotted line) in peripheral vascular resistance units (PRU)

minase In most cases it took at least one minute after the beginning of the histamine infusion until the effect of the histamine was clearly visible In many cases there was a tendency for the blood flow and the peripheral vascular resistance to vary considerably during the whole infusion period. (Fig 1 shows the response of forearm blood flow and of peripheral vascular resistance to histamine in one non-pregnant woman) The resting values of each individual were more constant

Table II gives the corresponding results for the pregnant women The effect of the infused histamine before inhibition of histaminase was with a few exceptions smaller than in the non-pregnant group After inhibition of histaminase however the effect of the infused histamine reached the non-pregnant level. The same fluctuations in the histamine effect were seen as in the non pregnant women

Table III shows the effect of infused histamine in the group of

Table II. Histamine Infusion in Pregnant Women

Case	Increase in Forearm Blood Flow before Inhibition of Histaminase	Decrease in Peripheral Vascular Resistance before Inhibition of Histaminase	Increase in Forearm Blood Flow after Inhibition of Histaminase	Decrease in Peripheral Vascular Resistance after Inhibition of Histaminase
1	0.66 (2.68 ± 0.16)	5.4 (36.4)	2.62 (4.63 ± 0.78)	7.8 (21.4)
2	1.03 (1.32 ± 0.34)	23.1 (69.4)	2.07 (1.59 ± 0.17)	27.8 (52.9)
3	0.22 (2.77 ± 0.21)	3.1 (33.8)	1.78 (2.52 ± 0.18)	14.6 (35.9)
4	1.36 (2.83 ± 0.75)	11.3 (32.1)	1.52 (3.36 ± 0.44)	7.2 (25.6)
5	0.57 (5.71 ± 0.60)	0.9 (15.3)	2.74 (4.78 ± 0.71)	5.7 (18.1)
6	0.40 (4.56 ± 0.96)	1.6 (15.3)	2.39 (4.58 ± 0.49)	4.4 (15.8)
7	0.00 (1.74 ± 0.23)	−1.1 (49.5)	0.62 (2.25 ± 0.24)	7.9 (37.4)
8	0.15 (1.94 ± 0.27)	1.7 (42.2)	1.74 (1.90 ± 0.10)	17.3 (43.1)
9	0.51 (2.65 ± 0.31)	5.7 (40.7)	1.59 (2.53 ± 0.25)	13.1 (42.5)
10	0.50 (3.84 ± 0.33)	1.8 (23.6)	0.89 (3.81 ± 1.10)	4.5 (23.2)
11	1.65 (2.30 ± 0.81)	11.2 (31.0)	2.84 (2.24 ± 0.51)	20.2 (36.4)
12	1.18 (5.47 ± 0.44)	2.9 (15.6)	4.08 (5.29 ± 0.76)	8.3 (17.9)
13	0.71 (3.04 ± 0.18)	3.8 (28.1)	2.57 (2.93 ± 0.75)	16.5 (33.3)
Mean	0.69	5.5	2.11	12.0

Infusion rate of histamine 12 micrograms per minute.
Forearm blood flow in ml/min × 100 ml
Peripheral vascular resistance in peripheral resistance units (PRU)
Figures in brackets indicate resting forearm blood flow and resting peripheral vascular resistance before histamine infusion.

pregnant women where histamine was infused on two successive occasions without interposed inhibition of histaminase. The average response to histamine was, if anything, slightly decreased during the second histamine infusion.

The effect of exogenous histamine, without inhibition of histaminase, in the non-pregnant group was compared with the corresponding results in the pregnant cases. The difference in response was tested for statistical significance by means of the Wilcoxon two-sample rank test. In Table IV the figures obtained by this test are shown. As the test variable the sum of the rank of the pregnant women was chosen. The increase in forearm blood flow was significantly less in the pregnant group. The peripheral vascular resistance did not fall as much in the pregnant as in the

Table III. *Pregnant Women Infused with Histamine on Two Successive Occasions*

Case	Increase in Forearm Blood Flow During the 1st Infusion	Decrease in Peripheral Vascular Resistance During the 1st Infusion	Increase in Forearm Blood Flow During the 2nd Infusion	Decrease in Peripheral Vascular Resistance During the 2nd Infusion
	2.28 (3.67 ± 0.85)	10.6 (27.1)	0.74 (3.05 ± 0.60)	5.3 (31.7)
	0.59 (2.18 ± 0.49)	9.4 (38.2)	0.25 (2.62 ± 0.23)	2.8 (29.5)
	2.66 (4.05 ± 0.38)	8.5 (19.9)	1.09 (3.92 ± 0.27)	4.4 (21.3)
	2.07 (2.34 ± 0.76)	*19.7 (40.3)*	*0.03 (4.19 ± 0.74)*	*−0.3 (22.0)*
	0.05 (1.54 ± 0.17)	1.0 (48.1)	0.57 (1.78 ± 0.33)	10.0 (43.1)
	0.02 (2.36 ± 0.29)	−2.2 (42.2)	0.16 (1.80 ± 0.52)	1.2 (56.4)
	0.82 (3.60 ± 0.39)	−0.7 (24.3)	1.79 (2.78 ± 0.31)	9.1 (31.4)
	2.33 (2.61 ± 0.58)	15.7 (34.0)	−0.29 (2.97 ± 0.99)	−2.5 (31.2)
Mean	1.35	7.8	0.54	3.8

Infusion rate of histamine 12 micrograms per minute.
Forearm blood flow in ml/min × 100 ml.
Peripheral vascular resistance in peripheral resistance units (PRU)
Figures in brackets indicate resting forearm blood flow and resting peripheral vascular resistance before histamine infusion.

non pregnant women The difference was however not statistically significant when the same testing method was applied.

If the change in response to histamine caused by inhibition of histaminase in the pregnant and in the non pregnant groups was compared there were as a rule higher values in the pregnant group The differences were tested for statistical significance by means of the Wilcoxon two-sample rank test. The figures of the test are given in Table V The sum of the rank of the pregnant women was chosen as the test variable The further increase in forearm blood flow and the corresponding decrease in peripheral vascular resistance caused by the inhibition of histaminase were significantly higher in the pregnant cases

Discussion

Venous occlusion plethysmography is a generally accepted method of determining blood flow per unit time per unit tissue It can also be repeated under strictly standardized conditions As de-

Table IV *Statistical Analysis of the Increase in Forearm Blood Flow and of the Decrease in Peripheral Vascular Resistance in Pregnant and Non-Pregnant Women During Intravenous Infusion of Histamine. The Wilcoxon Two-Sample Rank Test Was Used.*

	Forearm Blood Flow	Peripheral Vascular Resistance
Sum of rank of the pregnant women (21 cases)	308.5	318
Sum of rank of the non-pregnant women (13 cases)	286	277
Lower critical value for an one-tailed test on the 2.5 per cent level	312	312

The sum of the rank of the pregnant women shall be compared with the lower critical values

Table V *Statistical Analysis of the Potentiation of the Effect of Intravenously Infused Histamine on Forearm Blood Flow and on Peripheral Vascular Resistance Caused by Inhibition of Histaminase in Pregnant and Non-Pregnant Women. The Wilcoxon Two-Sample Rank Test was Used*

	Forearm Blood Flow	Peripheral Vascular Resistance
Sum of rank of the pregnant women (13 cases)	254	243.5
Sum of rank of the non-pregnant women (13 cases)	97	107.5
Upper critical value for an one-tailed test on the 2.5 per cent level	215	215

The sum of the rank of the pregnant women shall be compared with the upper critical values

monstrated by Grant and *Pearson* (1938) *Walden et al.* (1949) *Duff et al.* (1953 and 1954) and *Fox et al.* (1961) the blood flow is well suited to quantitative analysis of the effect of infused or injected vaso-active substances. In the present investigation the forearm blood flow and the peripheral vascular resistance were chosen as parameters of the effect of infused histamine. The fore-

Table III. Pregnant Women Infused with Histamine on Two Successive Occasions

ase	Increase in Forearm Blood Flow During the 1st Infusion	Decrease in Peripheral Vascular Resistance During the 1st Infusion	Increase in Forearm Blood Flow During the 2nd Infusion	Decrease in Peripheral Vascular Resistance During the 2nd Infusion
	2.28 (3.67 ± 0.85)	10.6 (27.1)	0.74 (3.05 ± 0.60)	5.3 (31.7)
	0.59 (2.18 ± 0.49)	9.4 (38.2)	0.25 (2.62 ± 0.23)	2.8 (29.5)
	2.66 (4.05 ± 0.39)	8.5 (19.9)	1.09 (3.92 ± 0.27)	4.4 (21.3)
	2.07 (2.34 ± 0.76)	19.7 (40.3)	0.03 (4.19 ± 0.74)	−0.3 (22.0)
	0.05 (1.54 ± 0.17)	1.0 (48.1)	0.57 (1.78 ± 0.33)	10.0 (43.1)
	0.02 (2.36 ± 0.29)	−2.2 (42.2)	0.16 (1.80 ± 0.52)	1.2 (56.4)
	0.82 (3.60 ± 0.38)	−0.7 (24.3)	1.79 (2.78 ± 0.31)	9.1 (31.4)
	2.33 (2.61 ± 0.58)	15.7 (34.0)	−0.29 (2.97 ± 0.99)	−2.5 (31.2)
ean	1.35	7.8	0.54	3.8

Infusion rate of histamine 12 micrograms per minute.
Forearm blood flow in ml/min × 100 ml.
Peripheral vascular resistance in peripheral resistance units (PRU)
Figures in brackets indicate resting forearm blood flow and resting peripheral vascular resistance before histamine infusion.

non pregnant women The difference was however not statistically significant when the same testing method was applied.

If the change in response to histamine caused by inhibition of histaminase in the pregnant and in the non-pregnant groups, was compared there were as a rule higher values in the pregnant group The differences were tested for statistical significance by means of the Wilcoxon two-sample rank test. The figures of the test are given in Table V The sum of the rank of the pregnant women was chosen as the test variable The further increase in forearm blood flow and the corresponding decrease in peripheral vascular resistance caused by the inhibition of histaminase were significantly higher in the pregnant cases.

Discussion

Venous occlusion plethysmography is a generally accepted method of determining blood flow per unit time per unit tissue It can also be repeated under strictly standardized conditions. As de-

Table IV Statistical Analysis of the Increase in Forearm Blood Flow and of the Decrease in Peripheral Vascular Resistance in Pregnant and Non-Pregnant Women During Intravenous Infusion of Histamine. The Wilcoxon Two-Sample Rank Test Was Used.

	Forearm Blood Flow	Peripheral Vascular Resistance
Sum of rank of the pregnant women (21 cases)	308.5	318
Sum of rank of the non-pregnant women (13 cases)	286	277
Lower critical value for an one-tailed test on the 2.5 per cent level	312	312

The sum of the rank of the pregnant women shall be compared with the lower critical values.

Table V Statistical Analysis of the Potentiation of the Effect of Intravenously Infused Histamine on Forearm Blood Flow and on Peripheral Vascular Resistance Caused by Inhibition of Histaminase in Pregnant and Non-Pregnant Women. The Wilcoxon Two-Sample Rank Test was Used.

	Forearm Blood Flow	Peripheral Vascular Resistance
Sum of rank of the pregnant women (13 cases)	254	243.5
Sum of rank of the non-pregnant women (13 cases)	97	107.5
Upper critical value for an one-tailed test on the 2.5 per cent level	215	215

The sum of the rank of the pregnant women shall be compared with the upper critical values.

monstrated by Grant and Pearson (1938), *Wakim et al.* (1949), *Duff et al.* (1953 and 1954) and Fox *et al.* (1961) the blood flow is well suited to quantitative analysis of the effect of infused or injected vaso-active substances. In the present investigation the forearm blood flow and the peripheral vascular resistance were chosen as parameters of the effect of infused histamine. The fore-

arm blood flow is less influenced by sensory stimuli than the blood flow of the hand (*Grant* and *Pearson* 1938) The forearm blood flow is largely skeletal muscle blood flow (*Grant* and *Pearson* 1938 *Allen Barcroft* and *Edholm* 1946) but it is impossible to deny that the effect of exogenous histamine observed in the present investigation also partly might depend on changes in the skin blood flow As the blood flow during infusion of histamine according to some investigators (*Walstm et al* 1949) seems to be different in the right and the left arm the blood flow determinations were always made in the right arm. The dose rate of 12 micrograms of histamine (histamine dihydrochloride) per minute was chosen for practical reasons Higher dose rates might give uncomfortable symptoms and thus disturb basal conditions. Lower dose rates give uncertain effects

The arterial blood pressure was fairly constant in most cases but small fluctuations of 5 to 10 mm Hg were observed. A changed form of the sphygmogram during the infusion of histamine may affect the calculation of the mean arterial blood pressure This fact however was judged to be of minor importance and the mean arterial blood pressure was calculated in the same way throughout the investigation

Using the forearm blood flow as a parameter of the effects of infused histamine the pregnant women were found to be less sensitive than the non pregnant subjects. The difference was statistically significant The cardio-vascular adaptations which occur during human pregnancy such as increase in cardiac output and rise in plasma volume (*Werkö Bucht Lagerlöf* and *Holmgren* 1948 *Kjellberg, Lönroth Rudhe* and *Sjöstrand* 1950 *Gemzell Robbe* and *Sjöstrand* 1954) are unlikely to explain this difference in histamine sensitivity

There was also a difference in peripheral vascular resistance during infusion of histamine between the pregnant and the non-pregnant women before inhibition of histaminase With the chosen level for statistical significance however this difference was not significant. After inhibition of histaminase, the effect of exogenous histamine was increased in the pregnant, but not in the non-pregnant group The differences in forearm blood flow and in peripheral vascular resistance were statistically significant.

The rate of blood flow is very variable and the experimental conditions were therefore standardized as much as possible. In no case there was any demonstrable signs of disturbed basal conditions. The magnitude of blood flow and of peripheral vascular resistance during rest corresponds fairly well with the results in pregnant and non-pregnant women reported by *Spetz* (1964).

That inhibition of histaminase in a few pregnant subjects did not appreciably increase the response to histamine was understandable. Aminoguanidine only inhibits the oxidative deamination of histamine and leaves the quantitatively important methylation of histamine intact (*Lindberg* and *Törnqvist* 1966).

Inhibition of histaminase alone did not increase the resting blood flow in either pregnant or non-pregnant women. The fact that there was no increase in resting blood flow in the pregnant women was interesting. As discussed in an investigation of the effect of exogenous histamine on the pulse rate in pregnant and non-pregnant women (*Törnqvist* 1968) the reason may be that there is no appreciable endogenous histamine production during human pregnancy or the production may be too small to give demonstrable effects. It is also possible that histamine produced in the foetus, unlike exogenous histamine is chiefly methylated (*Lindberg, Lindell* and *Westling*, 1963).

Histaminase inhibition has been proved to potentiate the effect of exogenous histamine in different animal investigations (*Lindell* and *Westling*, 1954 and 1956, *Westling*, 1956 and 1957, *Lindell* 1957). The results of the present investigation suggested that the increased plasma histaminase activity during human pregnancy might possibly reduce the response to exogenous histamine.

SUMMARY

1. The effect of an intravenous infusion of histamine on the forearm blood flow and on the peripheral vascular resistance was studied in 21 pregnant and 13 non-pregnant women. In order to investigate if the enzyme histaminase modified the effect of exogenous histamine the magnitude of the histamine response was studied before and after inhibition of this enzyme by means of an intramuscular injection of aminoguanidine sulphate.

arm blood flow is less influenced by sensory stimuli than the blood flow of the hand (*Grant* and *Pearson* 1938) The forearm blood flow is largely skeletal muscle blood flow (*Grant* and *Pearson* 1938 *Allen Barcroft* and *Edholm* 1946) but it is impossible to deny that the effect of exogenous histamine, observed in the present investigation also partly might depend on changes in the skin blood flow As the blood flow during infusion of histamine according to some investigators (*Wakim et al.* 1949) seems to be different in the right and the left arm the blood flow determinations were always made in the right arm. The dose rate of 12 micrograms of histamine (histamine dihydrochloride) per minute was chosen for practical reasons Higher dose rates might give uncomfortable symptoms and thus disturb basal conditions. Lower dose rates give uncertain effects

The arterial blood pressure was fairly constant in most cases, but small fluctuations of 5 to 10 mm Hg were observed. A changed form of the sphygmogram during the infusion of histamine may affect the calculation of the mean arterial blood pressure This fact, however was judged to be of minor importance and the mean arterial blood pressure was calculated in the same way throughout the investigation

Using the forearm blood flow as a parameter of the effects of infused histamine the pregnant women were found to be less sensitive than the non pregnant subjects. The difference was statistically significant. The cardio-vascular adaptations which occur during human pregnancy such as increase in cardiac output and rise in plasma volume (*Werkö Bucht Lagerlöf* and *Holmgren* 1948 *Kjellberg, Lönroth Rudhe* and *Sjöstrand* 1950 *Gemzell Robbe* and *Sjöstrand* 1954) are unlikely to explain this difference in histamine sensitivity

There was also a difference in peripheral vascular resistance during infusion of histamine between the pregnant and the non pregnant women before inhibition of histaminase. With the chosen level for statistical significance, however this difference was not significant. After inhibition of histaminase the effect of exogenous histamine was increased in the pregnant, but not in the non-pregnant, group The differences in forearm blood flow and in peripheral vascular resistance were statistically significant.

Acta physiol. scandinav 37 307 1956
Sperr S., Acta obst. et gynec. scandinav 43 309 1964
Törnqvist A. Acta obst. et gynec. scandinav to be published 1968
Unpublished
Walton K. G Peters G A. Terrier J C. and Horton B. T J Lab. Clin. Med 34 380 1949
Weiss, S Robb G P and Ellis, L B. Arch. Int. Med. 49 360 1932
Westling, H Acta physiol scandinav 38 91 1956
Acta physiol. scandinav 39 313, 1957
Acta physiol. scandinav 40 75 1957
Werkö L., Bucht H Lagerlöf H and Holmgren A. Nord. med. 40 1868, 1948

Received on Nov 14 1967

2. Exogenous histamine increased the forearm blood flow and decreased the peripheral vascular resistance in both pregnant and non pregnant women The effects of the infused histamine were more pronounced in the non pregnant cases The difference was statistically significant for the forearm blood flow

3 Inhibition of histaminase increased the response to exogenous histamine of both forearm blood flow and of peripheral vascular resistance significantly more in the pregnant women

Acknowledgements

This study was aided by grants from the Medical Faculty of the University of Göteborg The statistical treatment was performed by fil. mag Håkan Lindström.

REFERENCES

Allen W I Barcroft H and *Edholm O G* J Physiol. *105* 255 1946
Barcroft H and *Swan H J C* Sympathetic Control of Human Blood Vessels London 1953
Brownlee K. A. Statistical Theory and Methodology in Science and Engineering, New York 1961
Burn J H and *Dale H H* J Physiol *61* 185 1926
Duff F Greenfield A. D M Shepard J T and *Thompson I D* J Physiol. *120* 160 1953
Duff F and *Whelan R. F* Brit. J Pharmacol. *9* 413 1954
Folkow B Haeger K and *Kahlson G* Acta physiol. scandinav *15* 264 1948
Fox R. H Goldsmith R. Kidd D J and *Lewis G P* J Physiol. *157* 589 1961
Gemzell C A. Robbe H and Sjöstrand T Acta obst. et gynec. scandinav *33* 289 1954
Grant R T and *Pearson R. S B* Clin. Sci. *3* 119 1938
Green D G Lewis R. N Nickerson N D and *Heller A. L.* Am J Physiol. *141* 518 1944
Harmer I M and *Harris K. E.* Heart *13* 381 1926
Kjellberg, S R. Lönroth H Rudhe U and *Sjöstrand T* Acta med. scandinav *138* 421 1950
Kjellmer I and *Odelram H* Acta physiol. scandinav *63* 94 1965
Lindberg, S Lindell S E. and *Westling, H* Acta obst. et gynec scandinav *42* suppl. 1 35 1963
Lindberg, S and *Törnqvist A.* Acta obst. et gynec. scandinav *45* 131 1966
Lindell S E. and *Westling, H* Acta physiol scandinav *32* 230 1954

authors, however claim that the immediate increase in electrolyte excretion is transient, with a following rebound retention. This phenomenon is most pronounced for sodium, hence with protracted treatment the excretion of potassium will be relatively more increased than that of sodium. This is supposedly due to increasing aldosterone activity and may well explain the transitory diuretic effect of thiazides (*Peters* 1966 *Reid* 1962) Because of this aldosterone activity the risk of developing a sodium deficiency is negligible, but on the other hand it is known that protracted diuretic therapy may lead to hypokalaemia and hypochloraemic alkalosis. The changes in the potassium metabolism are not necessarily reflected in the serum potassium concentration. There is no definite correlation between the concentrations of potassium intra- and extracellularly This fact was illustrated by a report of 12 of 51 thiazide-treated women developing symptoms of hypokalaemia. In spite of "normal" serum potassium levels the symptoms were relieved by administration of potassium (*Barfield* and *Jungck* 1962) Further *Kraus et al.* (1966) noted a distinct fall in serum potassium concentration after prolonged thiazide treatment.

The aim of the present investigation was to observe the effects of thiazide treatment in pregnancy with a special reference to the serum electrolyte concentrations in parturients and foetuses and the correlation between the two. By the use of radio-isotopes it has been shown that at a molecular level water and sodium can move in either direction across the placenta, water moving more rapidly than sodium (*Reid* 1962) Therefore major differences between maternal and foetal serum electrolyte concentrations would not be expected. The placental barrier does not behave uniformly to drugs, but *Garnet* (1963) has demonstrated the placental transfer of chlorothiazide by detecting similar maternal and cord blood levels. In addition possible toxic effects on foetuses have been reported (*Rodriguez et al.* 1964)

Material and Methods

The material consists of 98 pregnant women with oedema alone or with pre-eclampsia. This was judged as mild in the presence

Gjønnæss, Halvard, Acta obstet. et gynec. scandinav. 47 404, 1968

From the Obstetrical Department (Hans Sundför M.D.) the County Hospital Lillehammer Norway

THIAZIDE TREATMENT IN PREGNANCY

With Special Reference to Maternal and Foetal Electrolytes

BY

HALVARD GJONNÆSS

It is well known that oedema is associated with sodium retention and treatment of pre-eclampsia and oedema of pregnancy with diuretics of the thiazide group has become increasingly common. In 1966 *Finnerty* and *Bepko* reported excellent results from prophylactic thiazide treatment The incidence of toxaemia in 1340 normal young pregnant women who had thiazide treatment was only 5 per cent, whereas in the untreated control group of 1743 patients it was 15 per cent. Similar results were reported by *Cuadros* and *Tatum* (1964) These results are contradicted by a report from Cleveland University (*Kraus et al.* 1966) A randomized double-blind investigation of continous prophylactic use of hydrochlorothiazide was undertaken on 1030 obstetrical patients. The incidence of toxaemia was not altered.

When pre-eclampsia is established the effect of the thiazides is still more uncertain. Most authors claim that the only effect of thiazides is to increase sodium excretion thereby decreasing the oedema without any apparent effect upon proteinuria or hypertension This effect can be blocked by sodium chloride (*Enger* 1966 *Jenssen et al* 1962 *Reid* 1962) The uncertainty of the effect of thiazide treatment and salt deprivation is further stressed by *Robinson* (1958) who reported excellent results from prophylactic salt administration—even the pre-eclamptic women improved

The effects of the various thiazides on the serum electrolyte concentrations show practically no differences

In 1956 *Tatum* and *Mulé* reported low serum sodium concentrations in toxaemic women who developed puerperal vasomotor collapse and they thought that a consistent salt restriction + thiazide treatment might lead to a low salt syndrome. Most

zides. Except for thiazides both groups received similar treatment, *i.e.* sedatives, bed rest and a modest salt restriction. The groups are not directly comparable with regard to parity and number of patients with severe pre-eclampsia. Between these two phenomena a correlation probably exists, pre-eclampsia being more frequent in primigravidae, therefore the influence of the degree of pre-eclampsia only will be discussed later. With regard to the other factors there is no obvious difference between the groups.

Maternal venous blood samples were taken for analysis during or immediately following delivery and on the 5th postpartum day. Foetal cord blood was collected immediately after delivery.

Serum concentrations of sodium and potassium were determined by flame-photometry (Evans Electroselenium Ltd., Flame Photometer Model A). The concentrations of chlorides were determined by titration with $Hg(NO_3)_2$ at a constant pH. By these methods the normal ranges are

Serum Na 137–147 mEq/l
Serum K 3.7–5 mEq/l
Serum Cl 96–106 mEq/l

Results

A. *Maternal effects*

The therapeutic effects were evaluated in two ways

1. The effects on clinical signs *i.e.* oedema, hypertension and proteinuria.
2. The effects on maternal and foetal serum electrolytes.

Concerning hypertension and proteinuria the two groups showed no difference. The beneficial therapeutic effect on oedema is demonstrated in Table II, which reveals regression, partial or complete in 74 per cent (39/53) in the T-group and in 16 per cent (7/45) in the C-group. This regression is most evident for patients with oedema only in the T group 96 per cent (23/24) and in the C-group 10 per cent (2/20). In the few cases of severe pre-eclampsia the effect on the oedema was questionable.

The effects of thiazide treatment upon the serum electrolyte concentrations were investigated in three ways

Table I

		T-Group 53 Pats	C-Group 45 Pats
Age	≤20 years	6	8
	21–30	31	23
	31–40	11	13
	41≤	5	1
Parity	para I (twin sets)	33(7)	15(1)
	II	6(1)	20
	III	6	7
	IV	5	3
	V	3	
Foetal weight	<2500 (twins)	8(6)	1
	2500–4000	32	30(2)
	4000<	16	15
Parturients with	oedema only	24	20
	moderate pre-eclampsia	20	24
	severe pre-eclampsia	9	1
Duration of symptoms	<1 week	3	15
	1–4 weeks	23	17
	4–8	14	8
	8<	13	5

of at least two of the following signs Oedema blood pressure 140/90–160/110 proteinuria <2 g/litre Esbach, and as severe if two of the following signs were present Oedema, blood pressure >160/110 proteinuria >2 g/litre Esbach.

The patients were divided into two groups one (T-group) was treated with thiazides (in most cases polythiazide (Renese®) 1 mg daily a few cases with hydrochlorothiazide hydroflumethiazide or tricholormethiazide) for varying lengths of time but always up to delivery All antenatal women with oedema or pre-eclampsia were given thiazides. The other group (C-group) served as controls. These were similar patients seen for the first time when admitted for delivery who had not been given thia-

Table III. *Serum Electrolytes mEq/l—Mean Values*

	Parturition		Puerperium	
	T-Group	C-Group	T-Group	C-Group
Serum-Na	135.3	138.1	138.6	139.0
Serum-K	3.87	4.24	4.22	4.45
Serum-Cl	101.9	104.3	101.7	103.6

For practical reasons it was not always possible to get serum-Na and -K both at delivery and in the puerperium. Consequently the groups are somewhat reduced, the T-group in the table consisting of 46 patients and the C-group of 42 patients for sodium; for potassium the T-group of 45 and the C-group of 40 patients.

concentrations below 3.5 mEq/l were found in 10 parturients in the T-group (19 per cent) and in 2 only in the C-group (4 per cent) and this difference is statistically significant ($p < 0.01$)

One patient had an exceptionally low serum-Na concentration.
She had been treated with strict salt restriction and polythiazide for 5 days before delivery. Her serum-Na was 116 mEq/l at delivery and 138 mEq/l in the puerperium.
In the cord blood sample it was 113 mEq/l. The concentrations of potassium and chlorides were normal.

The arithmetic mean values are shown in Table III. All the values except one were within the "normal" range; the mean serum-Na concentration for the T-group at delivery was below this and nearly significantly below the corresponding value for the C-group ($p < 0.05$). Also for potassium the lowest mean was found in the T-group at delivery, this being highly significantly ($p < 0.001$) lower than the corresponding value for the C-group.

In the puerperium, when the T-group had been without thiazide treatment for 5 days, there was a change in the serum electrolyte concentrations. This change is illustrated for each patient in Fig. 2, from which it is seen that for most of the patients the serum electrolyte concentrations in the puerperium were higher than at delivery. Of the 14 patients in the T-group with serum Na concentrations at delivery lower than 133 mEq/l, ten in the puerperium showed values above 135 mEq/l. In the C-group there was no parallel increase.

Table II. *Oedema—Effect of Treatment*

	Patients with					
	Oedema Only		Moderate Pre-Eclampsia		Severe Pre-Eclampsia	
Group	T	C	T	C	T	C
Progress or no change	0	11	3	13	5	1
Regression	23	2	12	5	4	0
Unknown	1	7	5	6	0	0
Total	24	20	20	24	9	1

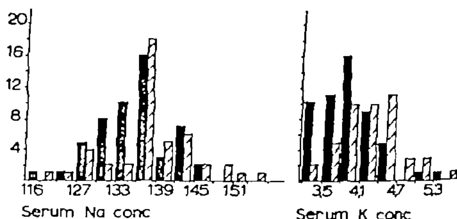


Fig. 1. Serum electrolyte concentrations in parturients. Ordinate: Number of patients. Abscissa: Serum electrolyte concentrations in mEq/l.
Thiazide group ■ Control group □

1. The extreme values.
2. The mean values.
3. The changes occurring after delivery.

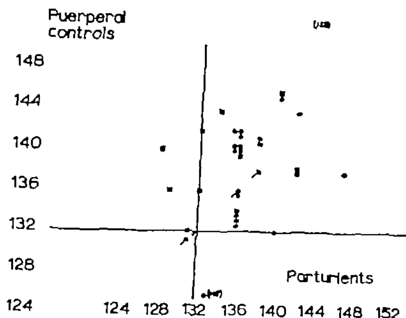
The distribution of serum-Na and serum-K-concentrations at delivery is shown in Fig. 1. At delivery more parturients in the T-group (28 per cent–15/53) showed serum-Na concentrations below 133 mEq/l than in the C-group (18 per cent–8/45). This difference is not statistically significant. For serum-K, however

Table IV Serum Electrolytes mEq/l—Mean and Extreme Low Values in Relation to Symptoms

	Group	Mean Values		Number of Parturients with		Total
		Na	K	Na ≤ 132	K ≤ 3.4	
Oedema	T	135.9	3.8	6	4	24
	C	137.6	4.3	4	1	20
Moderate pre-eclampsia	T	135.4	3.8	4	5	20
	C	138.9	4.2	3	1	24
Severe pre-eclampsia	T	133.4	4.2	5	1	9
	C	129.0	5.0	1	0	1
Total	T	135.3	3.87	15	10	53
	C	138.1	4.32	8	2	45

The mean increases in serum-Na concentrations were T-group 3.3 mEq/l C-group 0.9 mEq/l, the corresponding values for potassium were T-group 0.35 mEq/l C-group 0.21 mEq/l. The differences were not significant (Table III) It is seen that the mean concentrations of sodium in the puerperium were nearly identical in the two groups. For potassium the difference between the groups was reduced from 0.45 to 0.22 mEq/l. This difference is still nearly statistically significant ($p < 0.05$)

The results indicate that the T-group at delivery had lower serum-concentrations of sodium and potassium than the C-group. It is, however possible that this difference is due to factors other than the thiazide treatment. In an attempt to find some other reason, several factors were investigated. The duration of symptoms could not be shown to have any influence upon the serum electrolyte concentrations. Neither did the values depend upon the duration of treatment. The significance of the degree of toxaemia is demonstrated in Table IV. For sodium the lowest mean values and highest incidence of low serum concentrations were found in patients with severe pre-eclampsia. For patients with oedema or moderate pre-eclampsia the mean values for both sodium and potassium were lower in the T-group than in the



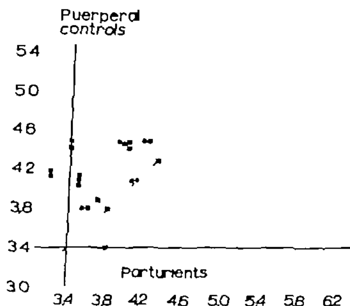


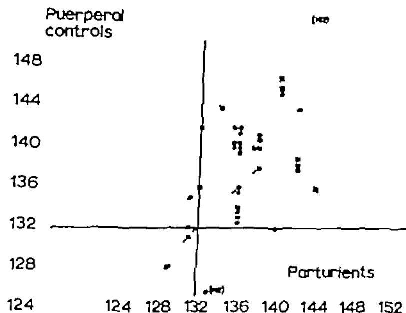
Fig 2 Serum electrolyte concentrations at delivery and 4-5 days after one symbol for each patient. The symbols on the diagonal represent patients who had the same serum electrolyte concentrations at delivery and in the puerperium. The symbols above the line indicate cases with an increase and those below cases with a decrease. Fig 2/1 Serum-Na concentrations mEq/l. Fig. 2/2: Serum-K concentrations mEq/l.
Thiazide group ■ Control group ○

Table IV Serum Electrolytes mEq/l—Mean and Extreme Low Values in Relation to Symptoms

	Group	Mean Values		Number of Patients with		Total
		N	K	$Na \leq 132$	$K \leq 3.4$	
Oedema	T	135.9	3.8	6	4	24
	C	137.6	4.3	4	1	20
Moderate pre-eclampsia	T	135.4	3.8	4	5	20
	C	138.9	4.2	3	1	24
Severe pre-eclampsia	T	133.4	4.2	5	1	9
	C	129.0	5.0	1	0	1
Total	T	135.3	3.87	15	10	53
	C	138.1	4.32	8	2	45

The mean increases in serum-Na concentrations were T-group 3.3 mEq/l, C-group 0.9 mEq/l, the corresponding values for potassium were T-group 0.35 mEq/l C-group 0.21 mEq/l. The differences were not significant (Table III) It is seen that the mean concentrations of sodium in the puerperium were nearly identical in the two groups. For potassium the difference between the groups was reduced from 0.45 to 0.22 mEq/l. This difference is still nearly statistically significant ($p < 0.06$)

The results indicate that the T-group at delivery had lower serum-concentrations of sodium and potassium than the C-group. *It is, however* possible that this difference is due to factors other than the thiazide treatment. In an attempt to find some other reason, several factors were investigated. The duration of symptoms could not be shown to have any influence upon the serum electrolyte concentrations. Neither did the values depend upon the duration of treatment. The significance of the degree of toxaemia is demonstrated in Table IV For sodium the lowest mean values and highest incidence of low serum concentrations were found in patients with severe pre-eclampsia For patients with oedema or moderate pre-eclampsia the mean values for both sodium and potassium were lower in the T-group than in the



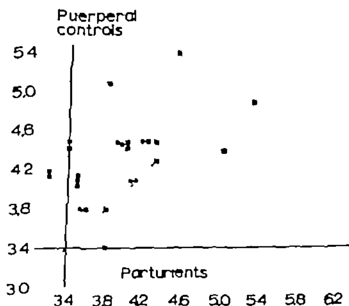


Fig. 2. Serum electrolyte concentrations at delivery and 4–5 days after: one symbol for each patient. The symbols on the diagonal represent patients who had the same serum electrolyte concentrations at delivery and in the puerperium. The symbols above the line indicate cases with an increase and those below cases with a decrease. Fig. 2/1: Serum-Na concentrations mEq/l. Fig. 2/2: Serum-K concentrations mEq/l.

Thiazide group ■ Control group ○

Table V Serum Electrolytes mEq/l—Mean Values

	Maternal		Foetal	
	T-Group	C-Group	T-Group	C-Group
Serum-Na	135 3	139.1	136.0	137.0
Serum-K	3 87	4.32	(5 03)	(5.28)
Serum-Cl	101 9	104 3	103 7	105.3

Numbers in parentheses refer to 30 patients in the T-group and 13 patients in the C-group

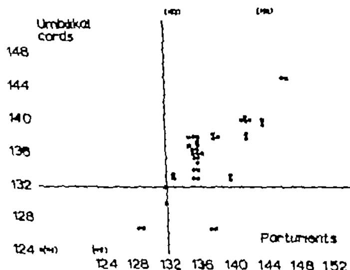


Fig 4 Serum sodium concentrations of the foetuses related to that of the mothers, one symbol for each pair The symbols on the diagonal represent cases with no difference between mother and foetus, the symbols below the line indicate babies with serum-N lower than that of their mothers, and vice versa The serum-Na concentrations in mEq/l.
Thiazide group ■ Control group ○

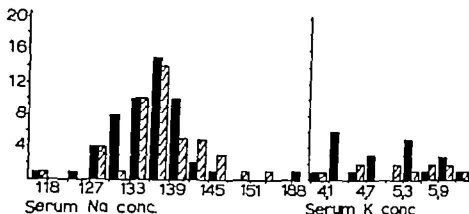


Fig 3 Serum electrolyte concentrations in blood from the umbilical cords. Ordinate: Number of patients. Abscissa. Serum-electrolyte concentrations in mEq/l.
Thiazide group ■ Control group ▨

C group and the incidence of low concentrations higher (for potassium 9/44 in the T-group and 2/44 in the C-group) It seems that patients with severe pre-eclampsia do have lower serum Na concentrations than others.

B *Foetal effects*

Clinical examination of the newborns showed no evident effects from the thiazide treatment There were no allergic or toxic effects no evidence of blood dyscrasias. There were no neonatal deaths.

The possible influence of thiazide treatment on foetal serum electrolytes *constitutes an important problem. Generally* the sodium concentrations in foetal serum closely follow those in maternal serum (Fig 4 compare also Figs 1 and 3) The mean values were similar (Table V) and extreme values follow the maternal pattern In the T-group 14 babies had serum Na con centrations under 133 mEq/l, in the C-group six *only In Fig.* 4 the distance from the diagonal shows directly the difference between maternal and foetal serum sodium concentration.

Of special interest are the serum Na concentrations in babies from parturients with low values, whether or not this is caused

Table V Serum Electrolytes mEq/l—Mean Values

	Maternal		Foetal	
	T-Group	C-Group	T-Group	C-Group
Serum-Na	135.3	138.1	136.0	137.0
Serum-K	3.67	4.32	(5.03)	(5.28)
Serum-Cl	101.9	104.3	103.7	105.3

Numbers in parentheses refer to 30 patients in the T-group and 13 patients in the C-group

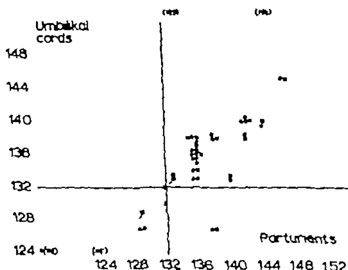


Fig. 4 Serum sodium concentrations of the foetuses related to that of the mothers, one symbol for each pair. The symbols on the diagonal represent cases with no difference between mother and foetus, the symbols below the line indicate babies with serum-Na lower than that of their mothers, and vice versa. The serum-Na concentrations in mEq/l.

Thiazide group ■ Control group ○

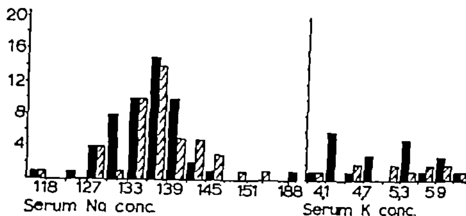


Fig 3 Serum electrolyte concentrations in blood from the umbilical cords. Ordinate Number of patients. Abscissa. Serum-electrolyte concentrations in mEq/l.
Thiazide group ■ *Control group* ▨

C-group and the incidence of low concentrations higher (for potassium 9/44 in the T-group and 2/44 in the C-group) It seems that patients with severe pre-eclampsia do have lower serum Na concentrations than others.

B *Foetal effects*

Clinical examination of the newborns showed no evident effects from the thiazide treatment There were no allergic or toxic effects no evidence of blood dyscrasias. There were no neonatal deaths.

The possible influence of thiazide treatment on foetal serum electrolytes constitutes an important problem. Generally the sodium concentrations in foetal serum closely follow those in maternal serum (Fig 4 compare also Figs. 1 and 3) The mean values were similar (Table V) and extreme values follow the maternal pattern in the T-group 14 babies had serum-Na concentrations under 133 mEq/l, in the C-group six only In Fig 4 the distance from the diagonal shows directly the difference between maternal and foetal serum sodium concentration

Of special interest are the serum-Na concentrations in babies from parturients with low values whether or not this is caused

As the T-group and the C-group were not quite comparable, other causes for the noted difference were sought. Parturients with severe pre-eclampsia had lower serum-Na concentrations than the less severe cases, as described by *Dieckmann* (1952) Reid (1962) and others. There was also a difference between the treated and untreated groups of patients with oedema only or with moderate pre-eclampsia.

For potassium the differences between the groups parallelled those for sodium, but there was no correlation with the severity of the toxaemia. Serum-K concentration below 3.5 mEq/l in 19 per cent of the T-group and in 4 per cent of the C-group is in accordance with the report from Cleveland University (*Kraus et al.*, 1966) where serum-K below 3.5 mEq/l was noted in 19.3 per cent of 506 thiazide-treated parturients and in 8.4 per cent of 524 controls ($p < 0{,}001$)

For chlorides there was no significant difference.

The correlation between maternal and foetal serum-Na concentrations was examined separately Women with high values seemed to deliver babies with lower values those with low serum-Na concentrations had babies with higher values. This was most evident in the T-group

Reports of osmotic pressure and electrolyte concentrations in parturients and their foetuses (*Altstatt* 1965 *Battaglia et al* 1960 Reid 1962) described the effects of radioisotopes or large olumes (3.5–5 litres) of glucose solutions. The observations indicate that a low osmotic pressure with low concentration of sodium in the extracellular fluid of parturients clearly leads to similar changes in the foetal extracellular fluid. Low serum-electrolyte-concentrations during pregnancy and also thiazide treatment, however represent a longer lasting influence. In consequence the organism has time to change its electrolyte metabolism. It has certainly been shown, that sodium traverses the placenta without difficulty but the passage of water is even quicker The present study indicates the possible existence of a mechanism of sodium retention in foetuses from mothers with a sodium deficiency independent of thiazide treatment.

Most authors agree that the declining natriuric effect of thiazide-treatment of long duration is due to increasing aldosterone-

Table VI. *Serum-Na of Foetus Minus Serum-Na of Mother mEq/l—Mean Differences*

	All Cases	Cases with Maternal	
		Na < 133	Na > 133
T-group (52 patients)	−0.37	+2.5	−1.4?
C-group (45 patients)	−1.2	+1.87	−1.86
T + C-groups (97 patients)		+2.27	−1.59

by thiazide treatment. The mean differences between the serum-Na concentrations of the babies and their mothers are shown in Table VI. Overall, and for parturients with serum-Na concentrations above 133 mEq/l, the foetal means were lower than the maternal. For parturients with low serum Na concentrations, however quite the opposite was the case, the serum-Na concentrations of the babies were higher than that of their mothers in both treated and control groups, this difference being statistically significant ($p < 0.01$) This phenomenon was most evident in the T-group (The case with a reading of 188 mEq/l was excluded, it would have made the difference even more pronounced)

The potassium determinations in cord blood samples were not reliable because of haemolysis which occurred in 23 cases in the T-group and in 32 cases in the C-group.

Discussion

The serum electrolyte concentrations of the parturients were influenced by thiazide treatment. The mean values the extreme values and the postpartum change all point to a depressive effect of thiazide treatment The postpartum sodium and potassium values did not change quite in parallel. Five days after delivery the difference between the serum-K concentrations of the T group and the C-group was still nearly significant (Table III) This might depend on differences in time necessary for the restoration of the body sodium- and potassium balance.

An exceptionally low serum-Na concentration was noted in one patient receiving thiazide treatment and strict salt restriction the postpartum rise was 22 mEq/l.

Conclusions

The effect of thiazide treatment on the oedema in pregnancy was excellent in patients with oedema only or a moderate degree of pre-eclampsia. In patients with severe pre-eclampsia the effect was insignificant. Effects on blood pressure or proteinuria were not observed.

Thiazide treatment may give a moderate decrease in the serum-Na concentration, and in combination with a strict salt restriction this decrease can be considerable.

The decrease in serum-K concentration was marked. Clinical signs of potassium deficiency were not observed.

The treatment had no obvious effect on the serum-Cl concentration

On the babies no adverse effects were seen. The serum-K could not be analysed because of haemolysis in a great number of cord blood samples.

When the maternal serum-Na concentration was higher than 133 mEq/l the serum-Na of the foetus was commonly lower than that of the mother On the other hand when the maternal serum-Na was below 133 mEq/l the foetal value was usually higher This preservation of foetal sodium was most pronounced in the thiazide treated group

SUMMARY

Partly contradictory reports on the value of thiazide treatment in pregnancy are discussed with special attention to possible untoward effects upon parturients or foetuses. 53 pregnant women treated with thiazides were compared with 45 similar patients not receiving thiazide treatment. The groups were comparable except that the treated group included more cases of severe pre-eclampsia than the control group

The effect of thiazide treatment upon oedema was excellent in patients with oedema only or with moderate pre-eclampsia. No effect was observed on hypertension or proteinuria.

Thiazide treatment was shown to give a moderate decrease in the serum concentration of sodium and a marked decrease in potassium concentration

activity *Benirschke et al.* (1956) have demonstrated that foetal adrenal glands contain small amounts of an aldosterone-like sodium-retaining factor as early as the ninth to the eleventh week of intrauterine life. In addition it is well known that steroids, whether of adrenal ovarian or placental origin, apparently readily traverse the placental membrane (*Snyder* and *Hoskins* 1962) The aldosterone activity in pregnancy is considered to be 2–10 times normal (*Reid* 1962) some of this steroid must be able to pass to and act on the foetus.

There are several possibilities concerning the site of action of the aldosterone. Although the renal tubules are anatomically well developed at birth they apparently have not acquired complete reabsorptive and excretory function. Also the filtration rate in the newborn is lower than that in the adult. The response of the kidney of the newborn to adrenocortical hormones is, however similar to that of the adult (*Klein* 1962) Another possibility is that aldosterone acts on the placenta, where most of the foetal excretion takes place *Berliner et al.* (1956) demonstrated several corticosteroids in the placenta including cortisone, hydrocortisone and aldosterone Whether the placenta has the ability to synthetize or interconvert adrenal steroids is still uncertain, but in an experimental work with laryngeal-carcinoma cells *Richards et al* (1966) demonstrated that aldosterone exerts a direct action on the normal electrolyte metabolism of unspecialised tissues.

Theoretically it should then be possible for the foetus to retain sodium in the event of sodium depletion in the mother for instance by the effect of aldosterone in the placenta.

The serum-K determinations in cord blood samples were rather unreliable because of the great number of haemolysed samples which can be attributed to the low mechanical resistance of foetal erythrocytes (*Michaëlson* and *Sjölin* 1965) From this series samples with visible haemolysis were excluded, but the high serum-K concentrations in the cord blood samples obviously indicate that some degree of haemolysis was present.

Richards P Smith K., Metcalfe-Gibson, A. and Wrong, O Lancet, II 1099 1966
Robinson, M., Lancet I 178 1958
Rodriguez S. U Leikin S. L and Hiller M. C., New Engl. J Med. 270 881 1964
Scribner B. H and Burnell J M., Metabolism, 5 468, 1956
Snyder F F and Hoskins, F M., in Reid, D E., A Textbook of Obstetrics, Saunders, Philadelphia and London, 81 1962
Tatum H J and Mulé J G Am. J Obstet. Gynec., 71 492, 1956

Received on Nov 1 1967

No adverse effects on the babies were seen. When the maternal serum-Na concentration was higher than 133 mEq/l, the serum-Na of the foetus was usually lower than that of the mother whereas when the maternal serum-Na concentration was under 133 mEq/l the foetal value was usually higher

Various possibilities as to the mechanism of this foetal sodium preservation are discussed.

Acknowledgements

I would like to thank miss Inger Norheim and the Laboratory Staff for performing the analyses.

The statistical calculations were done by amanuens Bjørn Birath, Statistiska Institutionen University of Lund.

Financial support has been gratefully received from Pfizer A/S, by dr Knut Graham Oslo

REFERENCES

Altstatt L. B. The Journal of Pediatrics *66* 985 1965

Barfield W E. and *Jungck E. C.*, J Med. Am. Georgia, *51* 538 1962

Battaglia F Prystowsky H Smisson C., Hellegers A. and *Bruns P* Pediatrics 25 2 1960

Bernirschke K. Block E. and *Hertig, A. T* Endocrinology *58* 598 1956

Berliner D L. Jones J E and *Salhanic H A.*, J Biol. Chem. *223* 1043 1956

Cuadros A. and *Tatum H J* Amer J Obstet. Gynec. *89* 891 1964

Dieckmann W J The Toxemias of Pregnancy C. V Mosby St. Louis, 115 *1952*

Enger E. T norske Lægeforening, *86* 1272, 1966

Finnerty F A. and *Bepko F J* JAMA, *195* 135 1966

G rnet J Obstet. Gynec. *21* 123 1963

Jenssen H Sommerfelt C and *Brodwall E. K.* T norske Lægeforening, *86* 916 1966

Johnson O D Ruchelman H and *Ford, R. V* New Engl J Med. 267 336 1962

Klein R. in Reid, D E., A Textbook of Obstetrics W B Saunders, Philadelphia and London 116 1962

Kraus G W Marchese J R. and *Yen S S. C.*, JAMA, *193* 128 1966

Michaëlson M and *Sjölin S.*, Acta paediat. Scand *54* 325 1965

Peters G in Gross F Antihypertensive Therapy Springer (Ciba Foundation) Berlin–Heidelberg, 32, 1966

Reid, D E. A Textbook of Obstetrics, Saunders, Philadelphia and London 759 1962

in the preceding one. Fresh untreated tissue from therapeutic abortions was used. The placentae were removed by minor Caesarean section. Termination of pregnancy was carried out on psychiatric and medico-social indications. These patients did not suffer from any somatic ailment.—The placentae were fixed in formol 5 per cent, before dissection. Frequently the dissection was carried out on fresh material and the specimens selected for study were then fixed in formol. The specimens were passed through 80 per cent alcohol, then successively through 96 per cent and absolute alcohol, each immersion was of 10 minutes duration. Finally the specimens were rinsed in two changes of xylol (each 10 minutes) and mounted in Eukitt. During the latter procedure the specimens were suitably positioned for study by using dissection needles under a stereo microscope.

Results

Each villous stem conveying the foetal blood vessels, arises from the chorionic plate as a trunk (*truncus chorii*) running into the intervillous space (IVS) where the stem divides into minor stems (*rami chorii*) which subdivide into minor ones (*ramuli chorii*). From these the terminal villi arise. Moreover villi can grow out anywhere on the chorionic tree and from the inside of the chorionic plate as well.

Most of the chorionic villi are oblong structures of varying length and thickness. The diameter of each villus however is rather constant. Numerous projections (sprouts) of varying length arise from the surface from short protuberances to long, slim structures. Syncytial buds appear on the surface. The buds grow to form twigs, which give the projections a ramified appearance. Grossly the villus appears not unlike a pinnate leaf (Fig. 1) —A considerable number of the villi are slightly swollen, likewise studded with projections (Fig. 2) —Using transillumination the projections may give the illusion of opacity (pseudo-opacity) but the villous tissue is not opaque.

This terminology (truncus, rami, ramuli) suggested by Bøe (1953) and, independently of him, by Wilkin (1954) seems to be widely accepted (cf. Boyd and Hamilton 1967).

Boe Finn, Acta obst et gynec scandinav 47 420, 1968

From the Department of Obstetrics and Gynecology (Finn Boe M.D.) Aker Hospital Oslo Norway

STUDIES ON THE HUMAN PLACENTA

II Gross Morphology of the Foetal Structures in the Young Placenta

BY

FINN BOE

Introduction

An extensive literature is available concerning the histology histochemistry and in more recent years the ultra structure of the human placenta However studies on the gross anatomy are scarce The comparative absence of published work in this field is rather surprising, since the foetal placenta is one of the most readily available structures for examination and yet it is one of the least known (*Crauford* 1962) Discrepancies in the result of histological studies may be due to deficient knowledge of the gross morphology because the various structures are not seen in their entirety in the histological preparation

Bumm (1890 1893) carried out dissection of the placenta for several years and he also quotes the preceding German authors in the field *Crauford* (1962) mentions the earlier Anglosaxon investigations This author developed an interesting technique the essence of which consists of a controlled digestion of the placenta with the proteolytic ferment trypsin *Alvarez* (1964) has studied the morphology by phase-contrast microscopy

So far the essential structure of the foetal placenta, the chorionic villus has been considered to be an anatomical entity During work with a dissection technique previously described (*Boe* 1967) it became apparent that the villi do not have a uniform appearance at all Accordingly it is intended in the present paper to give a description of the gross morphology of the chorionic villus and certain related structures in the foetal placenta.

Material and technique in the present study were similar to those

Thus, most of the villi, which may be considered the main type are of nearly uniform diameter or slightly swollen the surface studded with slim projections.

Two anatomical structures appear in striking contrast to the main type

1. Translucent villi
2. Syncytial bands

1. *Translucent villi* occur in considerable number everywhere in the IVS. The most characteristic feature of these structures is the lack of projections which makes their surface appear smooth and shining (Figs. 3 14 AB and 15) The lack of projections makes these structures markedly translucent to transillumination.

In their extreme form, and in transitional forms as well, these villi are markedly swollen, which causes their connection with the stem to appear narrowed. A short part of the connection, the neck appears narrowed, in contrast to the swollen corpus The villus thereby assumes the form of a cucumber (Fig. 4) The narrowing of the neck is not only relative as compared to the corpus, but also absolute in relation to the diameter of the main type of villus. Not infrequently the narrowed part appears elongated (Fig. 5)

All transitional forms occur from the main type of villus to the smooth, translucent villus. Bulb-shaped or oblong, swollen, translucent projections appear between the slim projections in the main type of villus (Fig 6) and syncytial bands as well (Fig 7) —Transitions from moderately swollen villi with a reduced number of projections (Fig. 8) to markedly swollen villi with short and plump (wartlike) projections (Figs. 9 and 4) are observed

The formation of cell islands (Boe 1967) seems to be due to changes in the translucent villus Proliferation of cytotrophoblastic cells occurs in a limited area (segment) of a villus. Rupture of the syncytial cover follows, and eruptions of cytotrophoblastic cell columns emerge into the IVS through very limited openings in the syncytial cover Cell columns from several villi fuse while forming a cell island.



Fig. 1 Chorionic villus *studded with projections of varying length*. Short syncytial band arises from the apex. 13 weeks. Transillumination. ×84

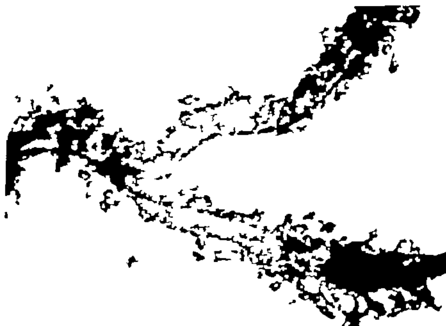


Fig. 2. Small villous stem subdivides into two slightly swollen villi studded with projections. 11–12 weeks. Transillumination. ×20

Thus most of the villi, which may be considered the main type are of nearly uniform diameter or slightly swollen, the surface studded with slim projections.

Two anatomical structures appear in striking contrast to the main type

1. Translucent villi
2. Syncytial bands

1 *Translucent villi* occur in considerable number everywhere in the IVS The most characteristic feature of these structures is the lack of projections which makes their surface appear smooth and shining (Figs. 3 14 AB and 15) The lack of projections makes these structures markedly translucent to transillumination.

In their extreme form, and in transitional forms as well, these villi are markedly swollen, which causes their connection with the stem to appear narrowed. A short part of the connection, the neck appears narrowed in contrast to the swollen corpus The villus thereby assumes the form of a cucumber (Fig. 4) The narrowing of the neck is not only relative as compared to the corpus but also absolute in relation to the diameter of the main type of villus. Not infrequently the narrowed part appears elongated (Fig. 5)

All transitional forms occur from the main type of villus to the smooth translucent villus. Bulb-shaped or oblong, swollen, translucent projections appear between the slim projections in the main type of villus (Fig. 6) and syncytial bands as well (Fig 7) – Transitions from moderately swollen villi with a reduced number of projections (Fig. 8) to markedly swollen villi with short and plump (wartlike) projections (Figs. 9 and 4) are observed.

The formation of cell islands (*Boe* 1967) seems to be due to changes in the translucent villus. Proliferation of cytotrophoblastic cells occurs in a limited area (segment) of a villus. Rupture of the syncytial cover follows, and eruptions of cytotrophoblastic cell columns emerge into the IVS through very limited openings in the syncytial cover Cell columns from several villi fuse while forming a cell island.



Fig 3 Markedly swollen villus with smooth surface. 13 weeks Dark field. ×84

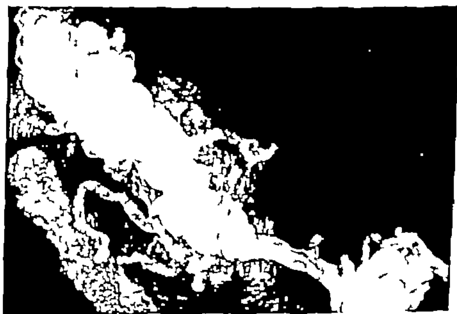


Fig 4 Markedly sw llen villus (cucumber shaped) arising from a small stem, with plump (wartlike) projections 13 week Dark field ×84



Fig 5 Proximal part of markedly swollen villus (right) with nearly smooth surface, the origin from the stem (left) is narrowed and elongated. 11 12 weeks Dark field. ×84



Fig 6 Three bulb-shaped and oblong swollen projections (above, left) between thin projections. 13 weeks Dark field ×84



Fig. 7. Long syncytial band (arrow) arises between slim projections. 11–12 weeks. Dark field. ×84



Fig. 8. Moderately swollen villus with a reduced number of projections. 11–12 weeks. Dark field. ×43



Fig. 9 Markedly swollen villus with plump projections. 13 weeks. Dark field. ×84

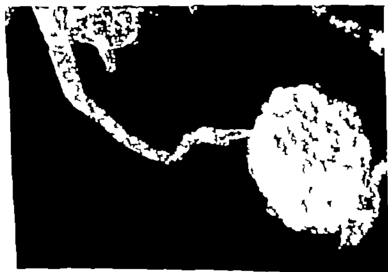


Fig. 10 Thin syncytial band arising from the apex of villus (above, left) attaching itself to cell island. 11 12 weeks Dark field. ×84



Fig. 7 Long syncytial band (arrow) arises between slim projections. 11–12 weeks. Dark field. ×84



Fig. 8 Moderately swollen villus with a reduced number of projections. 11–12 weeks. Dark field. ×43

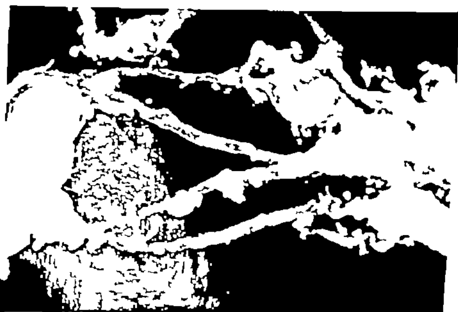


Fig. 12 Three translucent villi are closely related to cell island (centre) The intermediate villus is directly attached to the island, the other two are attached to it by syncytial bands.—The short syncytial bands from the lower villus attach themselves to cell island in neighbouring group (extreme left) 11 12 weeks. Dark field. 84

2. *Syncytial bands* likewise occur everywhere in the IVS forming a framework (Gerlist *Hörmann* and *Lemtis* 1965) of communications. These structures may form short communications between the villi (bridges) but they may also achieve a con siderable length (bands) In their most pronounced form the bands appear as very thin, threadlike structures (Fig 10) Most frequently the bands arise from the distal (apical) part of the villi (Figs. 11 AB 12 14 AB and 15) Usually the band narrows during its further course In the proximal thicker parts near its origins, a small number of short projections are normally found whereas the distal threadlike part usually appears smooth, with out projections. Most of the syncytial bands attach themselves to cell islands (Figs. 10 11 AB 12 and 16) Syncytial bands from different groups of villi attach themselves to intermediate cell islands (Fig. 12) but the bands may form communications be tween the same group of villi as well (Figs. 7 and 14 AB)



A



B

Fig. 11. *A.* Terminal branch with numerous projections (right) swells and subdivides into translucent swollen villi from the apical parts of which syncytial bands with short bud-shaped projections arise and attach themselves to a cell island (dark opaque). 11 12 weeks. Transillumination ×20. *B.* Detail of Fig. 11 A. Dark field. ×84



Fig 12 Three translucent villi are closely related to a cell island (centre) The intermediate villus is directly attached to the island, the other two are attached to it by syncytial bands —The short syncytial bands from the lower villus attach themselves to cell island in neighbouring group (extreme left) 11 12 weeks Dark field. 84

2. *Syncytial bands* likewise occur everywhere in the IVS forming a framework (Gerstl *Hörmann* and *Lemtis* 1965) of communications. These structures may form short communications between the villi (bridges) but they may also achieve a considerable length (bands) In their most pronounced form the bands appear as very thin, threadlike structures (Fig. 10) Most frequently the bands arise from the distal (apical) part of the villi (Figs. 11 AB 12 14 AB and 15) Usually the band narrows during its further course In the proximal thicker parts near its origins a small number of short projections are normally found, whereas the distal threadlike part usually appears smooth without projections Most of the syncytial bands attach themselves to cell islands (Figs 10 11 AB 12 and 16) Syncytial bands from different groups of villi attach themselves to intermediate cell islands (Fig 12) but the bands may form communications between the same group of villi as well (Figs. 7 and 14 AB)

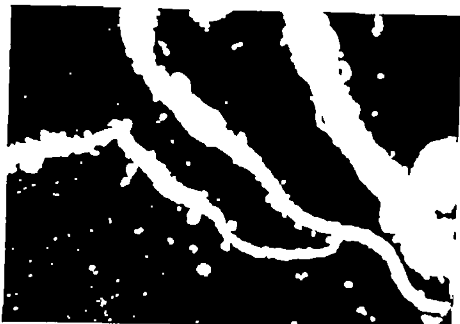


Fig 13 Three long slim villi of varying thickness with few and short (bud-shaped) projections 11–12 weeks. Dark field. ×84

Again it must be emphasized that there are numerous transitional forms from the main type of villus to the final extremity i e the smooth threadlike syncytial band A common finding is a long slim villus of varying diameters but markedly slimmer than a villus of the main type with only a few short projections on its surface (Figs. 13 and 15)

Probably the most striking feature of the gross morphology is the vast variety of the various fetal structures. This variability is due to the transitional forms but especially to the fact that the latter occur in innumerable combinations Most frequently the translucent villus is cucumber shaped but translucent structures may also appear as shorter cylinders spherical or globular structures usually interposed between syncytial bands (Fig 16) —The translucent villi and the syncytial bands most often appear in close relation to the cell islands (Figs 10 11 AB and 16) and to the inside of the basal plate

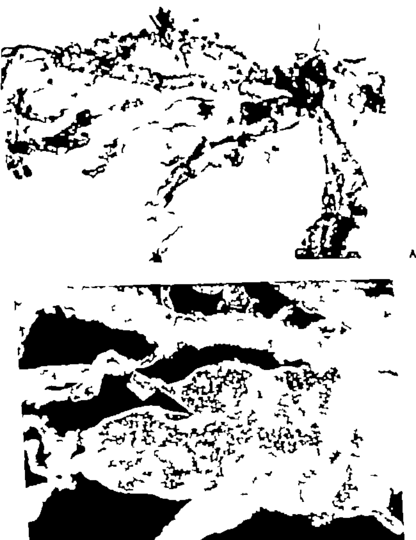


Fig 14 *A* A terminal branch (above right) subdivides into two villi with numerous projections Marked terminal swelling of the upper villus, from the apical part of which number of syncytial bands arise —Centre, below Three translucent villi with smooth surface Syncytial bands arise from the apical parts Right Markedly swollen illus with reduced number of projections. Arrow Origin of long syncytial band 11 12 weeks. Transillumination 43 B Detail of the translucent villi Dark field 84

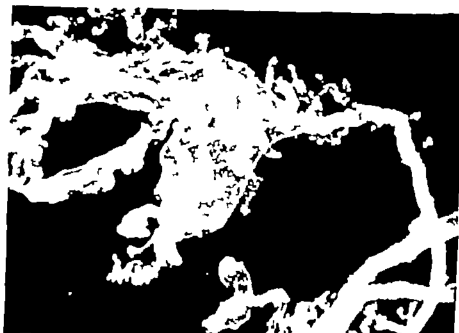


Fig. 15. Long, slim villus with very few bud-shaped projections swells terminally into a translucent structure from which a number of syncytial bands arise. 11-12 weeks. Dark field. ×84



Fig. 16. Translucent structures and syncytial bands in relationship to a cell island (centre dark opaque). 10 weeks. Untreated specimen. Transillumination.

Opaque Tissue

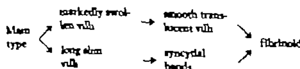
On transillumination the appearance of the cell islands varies from light to almost black opaque. Histological evidence suggests that the increasing opacity is due to the gradual transformation of the cell islands into fibrinoid (Boe 1967) This variability however does not appear in the basal plate which is uniformly and moderately opaque —In addition to the cell islands and the basal plate, fragments of opaque tissue are a constant finding in the foetal placenta.

Discussion

Most chorionic villi are oblong structures of uniform thickness or slightly swollen Their surface is studded with slim projections. This appearance may be considered characteristic of the main type of villus.

Numerous transitional forms lead to two secondary anatomical structures (1) markedly swollen, smooth, translucent villi without projections presumably the result of oedema in the stroma, and (2) thin, smooth, threadlike syncytial bands also without projections.

These structures must both be considered derivatives of the main type of villus. The numerous transitional forms support this view Their formation may be explained in two different ways 1) The main type primarily may become altered into a translucent villus and the formation of the syncytial band may follow secondarily from this structure. 2) The main type may become altered into the two secondary structures *via* two different pathways each with its transitional forms.—The latter possibility seems more likely on the evidence available even if the former possibility can not be excluded.



Opaque Tissue

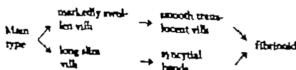
On transillumination the appearance of the cell islands varies from light to almost black opaque. Histological evidence suggests that the increasing opacity is due to the gradual transformation of the cell islands into fibrinoid (Boe 1967). This variability however does not appear in the basal plate which is uniformly and moderately opaque.—In addition to the cell islands and the basal plate fragments of opaque tissue are a constant finding in the foetal placenta.

Discussion

Most chorionic villi are oblong structures of uniform thickness, or *slightly swollen. Their surface is studded with slim projections* This appearance may be considered characteristic of the main type of villus.

Numerous transitional forms lead to two secondary anatomical structures, (1) markedly swollen smooth, translucent villi without projections presumably the result of oedema in the stroma and (2) thin smooth threadlike syncytial bands also without projections.

These structures must both be considered derivatives of the main type of villus. The numerous transitional forms support this view Their formation may be explained in two different ways 1) The main type primarily may become altered into a translucent villus and the formation of the syncytial band may follow secondarily from this structure. 2) The main type may become altered into the two secondary structures *via* two different pathways each with its transitional forms.—The latter possibility seems more likely on the evidence available even if the former possibility can not be excluded.



The term main type" is preferred to omit the term normal type because the *secondary structures, in my consideration* are normal structures —Two objections may be raised The translucent villus may be regarded as (1) a pathological structure (2) a fixation artefact —However the material was obtained from patients with mental ailments None of these patients suffered from any somatic disease Furthermore in my experience the translucent villus is readily demonstrable in fresh untreated placental tissue immersed in physiological saline solution. The structure undergoes very little change during fixation.—Moreover translucent villi and villi of the main type are frequently found side by side connected with a common stem (Fig 14 AB)

The morphological differences between the main type of villus on the one hand and the two secondary structures on the other *may be explained by two metabolic processes* anabolic and catabolic diametrically opposed but running simultaneously The projections are considered to be the anatomical manifestation of the intensive growth energy in the young placenta *i.e.* anabolic processes

The two secondary structures the translucent villus and the syncytial band provide a striking contrast to the main type of villus The formation of these structures may be interpreted as the result of *catabolic* processes *i.e.* they are worn-out tissues *which undergo a gradual regressive change until they are trans* formed into its final state fibrinoid mainly in the cell islands and the basal plate Accordingly these altered structures chiefly occur in close relationship to the cell islands and the basal plate

The projections presumably the result of great proliferation appear to be the most highly differentiated structures in the young placenta The occurrence of bulb-shaped translucent projections is thought to represent incipient regressive changes. The considerable number of altered villi either with a reduced number of projections or *completely lacking them, may* be evidence that villi have a limited life span —Replacement of worn-out tissue and the natural growth of the chorionic tree occur by the free outgrowth of new villi or perhaps more likely by longitudinal more or less dichotomous division of the branches, as suggested by *Boyd* and *Hamilton* (1967)

The fundamental role of the circulatory system in this process will be discussed in a paper now in preparation.

SUMMARY

1. The majority of the chorionic villi are oblong structures of uniform diameter or slightly swollen their surfaces studded with slim projections.
2. Via numerous transitional forms two secondary anatomical types are formed,
   (a) markedly swollen, translucent (oedematous) villi without projections
   (b) very thin threadlike syncytial bands without projections forming a framework of communications in the inter villous space.
3. The translucent villi and the syncytial bands occur in close relationship to the cell islands and the basal plate

Acknowledgements

The Author wishes to express his sincere gratitude to Elizabeth M. Ramsey M. D for most valuable advice and criticism.—The photographs were taken by Tore Hagen, M. D Dept. of Pathology (Head K. A. Larsen, M. D)

REFERENCES

Alvarez H Obst Gynec. 23 813 1964
Boyd, J D and Hamilton W J J Obstet. Gynaec. Brit. Cwlth., 74 161 1967
Barnum E Arch Gynäk 37 1 1890
Ibid 43 181 1893
Bøe F Acta obst et gynec scandinav 32 suppl. 5 1953
Ibid 46 591 1957
Crawford J M Am J Obst Gynec. 84 1543 1962
Hörmann G and Lemtis, H in Schwalm-Döderlein, Klinik der Frauenheilkunde und Geburtshilf III 425 1965 Urban u. Schwarzenberg
Wilkin P Gynec et obst 53 239 1954
— Le Placenta Humain Masson, 1960

Received on Oct 6 1967

Jensen H Lyngbye J and Davidsen S Acta obst et gynec. scandinav 47 [illegible] 196[illegible]

From the Medical Department (K Kjerulf M.D) the Department of Clinical Chemistry (S Müllertz M.D) Blegdamshospitalet Copenhagen and the Department of Obstetrics and Gynaecology (H G Berthelsen) Kommunehospitalet Aalborg, Denmark

HISTOCHEMICAL INVESTIGATION OF THE THERMOSTABLE ALKALINE PHOSPHATASE IN THE NORMAL FULL TERM PLACENTA

BY

H JENSEN J LYNGBYE AND S. DAVIDSEN

The presence of alkaline phosphatase in blood was first reported by *Demuth* (1925 a and b) Subsequently it has been demonstrated that levels of serum alkaline phosphatase are increased to almost twice the normal non pregnant values in women examined during the third trimester of pregnancy Recent evidence supports the view that the increased phosphatase activity is due to a heat stable enzyme produced in the placenta (*Lyngbye* and *Christoffersen* 1968) This evidence is based on the fact that the heat stability of the alkaline phosphatase from placental tissue is shared by about half of the serum phosphatase activity in late pregnancy that the heat-stable serum enzyme fraction has the same electrophoretic mobility and the same stability against EDTA inactivation as the placental phosphatase and that the alkaline phosphatase in maternal sera at term is partially inactivated by an anti human placental alkaline phosphatase antibody from rabbits

The purpose of this work is to investigate histochemically the thermostability of the alkaline phosphatase directly in the placental tissue

EDTA = Ethylene diamine tetraacetic acid.

Materials

Placentas were obtained immediately after delivery from 71 normal healthy women (age 17–39 years) who had had an uncomplicated full term pregnancy Two of the subjects were delivered by Caesarean section because of a narrow pelvis and from these women tissue samples were also obtained from the liver striated muscle (abdominal musculature) smooth muscle (myometrium) and intestinal mucosa (jejunum) The intestinal biopsies were taken 1–2 weeks before delivery All the tissue samples were treated in the same way (see below)

Blood samples were obtained by venipuncture immediately before delivery from 39 of the patients and from 13 of these women during the last trimester as well. Determinations of the thermostable serum alkaline phosphatase activity were made according to *Lyngbye* and *Christoffersen* (1968)

Methods

Alkaline phosphatases in the tissue samples were demonstrated with the azo dye method described by *Pearse* (1961)

Two to four biopsies (approximately 4 mm × 4 mm) were taken from each placenta immediately after delivery Samples were taken from marginal as well as central areas and fixed at 4 C for 20 minutes in a 10 per cent (v/v) solution of formalin containing 1 per cent (w/) calcium chloride and 1 per cent (w/) calcium carbonate The fixative was removed with a 1 per cent (w/v) solution of gum arabic, which was also used for storage of the biopsies The samples were then frozen with carbon dioxide—acetone and sliced in a cryostat (Pearse SLEE) Sections (8) from each preparation were incubated with the substrate for 5 10 and 20 minutes respectively Some were subjected to preliminary heating (see below) After this procedure a reddish-brown precipitate was seen microscopically in all

15 mg sodium-α-naphthyl phosphate dissolved in 20 ml 0 1 m tris buffer solution, 20 mg Fast Red TR (5-chloro-2-diazo-toluene) was added. The substrate had a pH of 8 9 (20° C)

cells containing alkaline phosphatase The preparations wer trast stained with hematoxylin

To investigate the thermostability of the alkaline phosp tissue slices from each sample were heated for 30 minute 0 01 M magnesium chloride solution at 56 C or 70 C procedure was performed just before incubation with th strate Other tissue slices were placed in the magnesium ch solution for 30 minutes at room temperature (approxi 20 C) or incubated immediately after cutting with or w sodium-α naphtyl phosphate added (control preparations make sure that the alkaline phosphatase did not escape int magnesium chloride solution this was examined after us showed no alkaline phosphatase activity Furthermore slice ped in magnesium chloride solution and kept dry (vapour sa ed atmosphere) in an oven for 30 minutes at 56 C or 70 not show any difference microscopically from correspondin parations heated in magnesium chloride solution (see above

Samples from other tissues (liver muscle intestine) treated in the same way The liver slices were however cubated with the substrate for 1–3 hours

Results

In the placentas the alkaline phosphatase activity was found in the syncytiotrophoblast of the chorionic villi (Fig 1) and unchanged after heating at 56 C and 70 C for 30 minutes (2) It was noted that the activity was localized mainly in outer cell borders and to a lesser extent in the areas in wh the cells were attached to the connective tissue stroma of villi No reaction was seen in the stroma. The activity of syncytiotrophoblast was not affected when applied to EDTA the magnesium chloride solution for 30 minutes at room tempe ture before incubation with the substrate (10 cases) The c trol preparations (no sodium-α naphthyl phosphate added) sho ed no activity in the syncytiotrophoblast or elsewhere

Disodium EDTA added to a final concentration of 0 01 M.



Fig. 1 Alkaline phosphatase in normal term placenta. Unheated preparation, placed in 0.01 M magnesium chloride solution at room temperature for 30 minutes before incubation with the substrate. The main reaction is seen in the outer cell borders of the syncytiotrophoblast and a weaker reaction is noted in inner cell borders. Stroma and blood vessels negative. (×450)



Fig. 2 Alkaline phosphatase in normal term placenta. Preparation heated at 70 C for 30 minutes before incubation with the substrate. Reaction unchanged compared with the preparation shown in Fig. 1 (×450)

In the 39 cases in whom the thermostable serum alkaline phosphatase was also determined, the results confirmed the findings of *Lyngbye* and *Christoffersen* (1968)

The activity in the intestinal mucosa was localized to the surface epithelium and the small vessels in the submucosa (Fig.



Fig 3 Alkaline phosphatase in intestinal mucosa (jejunum) from a normal woman in late pregnancy Unheated preparation The reaction is localized t the surface epithelium and the small vessel in the submucosa (×450)



Fig 4 Intestinal mu osa (j junum) from a normal woman in late pregnancy (from the same tissue sample a i Fig 3) Preparation heated at 70 C for 30 minutes before incubation with the substrate No reaction is seen. (×450)

3) and disappeared completely after heating at 70 C (Fig 4) or addition of EDTA Only a slight decrease was seen after heat ing at 56 C

In the liver both the parenchymal cells and the vessels reacted, but in striated and smooth muscle this was only found in the

arterioles and capillaries. All these reactions were lost after application of heat or EDTA.

Discussion

The localization of the alkaline phosphatase activity to the syncytiotrophoblast confirms earlier histochemical observations (*Dempsey* and *Wislocki* 1945 *Wislocki* and *Dempsey* 1946 *Botella* and *Cano* 1950 *Thomsen* 1955 *Dumont* 1957 *McKay et al.* 1958 Thomsen, 1958 *Ahmed* and *King*, 1959 *Wielenga* 1962 *Wielenga* and *Willighagen* 1962 *Wachstein et al.*, 1963 *Curzen* 1964) but no investigation of the heat-stability using this technique has been reported.

The alkaline phosphatase in placental tissue was the only one found to be entirely stable against heat and EDTA inactivation. This confirms the findings in the investigations on homogenates of placental (*Neale et al.* 1965) and other tissues (*cf Lyngbye* and *Christoffersen* 1968) These results when considered with the changes in maternal serum alkaline phosphatase in late pregnancy and the heat-lability of this enzyme in foetal blood strongly support the theory that the increase in the serum alkaline phosphatase activity in the third trimester is due to an enzyme produced in the placenta (*cf Lyngbye* and *Christoffersen* 1968)

SUMMARY

Using a histochemical technique the heat-stability of the alkaline phosphatase in 71 normal full term placentas was investigated. It was demonstrated, that the placental phosphatase was the only stable against heat at 70 C for 30 minutes. This fact together with earlier biochemical observations on the alkaline phosphatase in maternal blood, foetal blood, and placental tissue strongly supports the theory that the increased serum alkaline phosphatase values in late pregnancy are due to production of this enzyme in the placenta.

Acknowledgements

This study was aided by grants from Kong Christian den Tiendes Fond, Statens Almindelige Videnskabsfond, and P Carl Peter

sens Fond Overlæge dr med. H O Bang and overlæge dr med. E. Hess Thaysen, Aalborg Kommunehospital are thanked for kind assistance

REFERENCES

Ahmed Z and *King, E. J* Biochim biophys Acta 34 313 1959
Botella J and *Cano A.* Arch med exp (Madrid) 13 45 1950
Curzen P J Obstet. Gynaec. Brit. Cwlth 71 388 1964
Dempsey E. W and *Wislocki G B* Amer J Anat. 76 277 1945
Demuth F Biochem. Z. 159 415 1925 (a)
– Biochem Z. 166 162, 1925 (b)
Dumont M Presse méd (Paris) 65 535 1957
Lyngbye J and *Christoffersen J B* Dan. med Bull 15 13 1968
McKay D G Hertig, A T Adams E C and *Richardson M V* Obstet. Gynec 12 1 1958
Neale F C. Clubb J S Hotchkis D and *Posen S* J clin. Path. 18 359 1965
Pearse A. G E. Histochemistry 2. ed. J & A. Churchill London 1951
Thomsen K. Arch Gynäk 187 1 1955
– Geburtsh Frauenheilk 18 354 1958
Wachstein M Meagher J G and *Ortiz J* Amer J Obstet Gynec. 87 13 1963
Wielenga G Nederl T Verlosk Gynaec. 62 450 1962
Wielenga G and *Willighagen R. G J* Amer J Obstet Gynec. 84 1059 1962
Wislocki G B and *Dempsey E. W* Endocrinology 38 90 1946

Received on Oct 5 1967

Stenström, Bengt, Acta obst. et gynec. scandinav. 47 443, 1968

From the Department of Obstetrics and Gynaecology (H. Jedberg, M.D.)
Regionsjukhuset Örebro Sweden

ORAL CONTRACEPTION USING A SEQUENTIAL METHOD

A Clinical Investigation

BY

BENGT STENSTRÖM

Since *Pincus* in 1955 introduced oral contraception with synthetic hormones the development and use of these preparations have greatly increased. Already at an early stage it was evident that this method meant, practically speaking, absolute security. In one of the first reported investigations (*Pincus et al.* 1958) the number of births was diminished by 98 per cent. It is highly probable that the pregnancies that did occur were due to negligence in taking the tablets.

The use of contraceptive tablets during the last 10 years has fulfilled the expectations that *Pincus* entertained.

Different methods of oral contraception

At present two different regimes are used to prevent pregnancy by inhibiting ovaluation, namely the combined and the sequential method. Recently a third method has also been tested where a low dose of a gestagenic substance is administered daily. In the latter method the preventive effect may be explained partly by inhibition of ovaulation and partly by action on the cervical mucous so that the passage of the spermatozoa is inhibited.

Combined method

In this regime two hormones are employed, oestrogenic and gestagenic. The first preparations used belonged to this group and

contained comparatively high doses of hormonal substances. Subsequently is was found that the dose could be reduced whilst retaining the antiovulatory effect

Sequential method

In this method oestrogenic hormone is administered for about two weeks *after which a combination of oestrogenic and gestagenic* substances is given so that the total period of administration is 20–21 days Bleeding, similar to menstrual bleeding, usually occurs within 5 days after discontinuing the combined tablets. It is considered that in this way a cycle is obtained more closely resembling the normal *Another advantage associated with the sequential* method is that much smaller amounts of hormones need be administered and possibly a lower frequency of side effects can be expected. Since oestrogenic hormone is given alone during the first part of the cycle for the sake of security a somewhat larger dose than that contained in the usual combined preparations is used Thus with combined therapy 0 05 mg of ethinyl oestradiol or mestranol is sufficient, whereas in the sequential regime 0 08–0 1 mg is required.

Physiologically the combined method differs from the sequential method inasmuch as in the former both the proliferative and the secretory phases are much shortened and followed by a pseudodecidual phase that completes the cycle When the sequential method is used the pseudodecidual phase is absent, but instead the proliferative phase is prolonged and the secretory phase shortened in relation to the course of the normal cycle The sequential method is regarded as an advance in development and signifies less interference with the natural menstrual pattern The menstrual bleeding proceeds from a more natural mucous membrane which explains why as a rule it is not so greatly reduced as in the combined method.

Clinical trials with the sequential method

Goldzieher (1964) has reported on over 15 000 well observed cycles with the sequential method 15+5 and has used a prepara

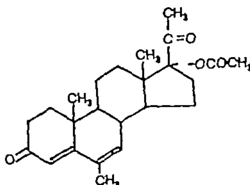


Fig. Megestrol acetate

tion consisting of 15 tablets each containing 0.08 mg of mestranol, and 5 tablets each containing 0.08 mg of mestranol + 2 mg of chlormadinone. In his investigation this combination was completely effective. *Goldzieher* considers that the sequential method will be widely employed in the future as the total monthly progesterone content is only 3/4 of that in the combined regime. Other regimens have also been tested, for example, 14–6 (*Tyler* 1966) and 11–10 (*Mears* 1964)

Own investigation

At the Gynaecological Clinic in Örebro a new sequential preparation has been tested under the name of Ovisec® Each package contains 16 red tablets, each comprising 0.1 mg of ethinyloestradiol, 5 white tablets each comprising 0.1 mg of ethinyloestradiol 1.0 mg of megestrol acetate and 7 blue tablets with lactose (= placebo tablets) Megestrol acetate is a 17 α-hydroxyprogesterone derivative, Fig. 1

Dosage According to the enclosed directions, on the 5th day of menstruation the patient has to start taking the red tablets daily and then continue with the white and the blue tablets. A new package is begun without any interval.

The preparation has been developed by The British Drug House Ltd London, and kindly supplied by AB Hässle Göteborg, Sweden

contained comparatively high doses of hormonal substances. Subsequently is was found that the dose could be reduced whilst retaining the antiovulatory effect

Sequential method

In this method oestrogenic hormone is administered for about two weeks after which a combination of oestrogenic and gestagenic substances is given so that the total period of administration is 20–21 days Bleeding, similar to menstrual bleeding, usually occurs within 5 days after discontinuing the combined tablets It is considered that in this way a cycle is obtained more closely resembling the normal Another advantage associated with the sequential method is that much smaller amounts of hormones need be administered and, possibly a lower frequency of side effects can be expected. Since oestrogenic hormone is given alone during the first part of the cycle for the sake of security a somewhat larger dose than that contained in the usual combined preparations is used Thus with combined therapy 0 05 mg of ethinyl oestradiol or mestranol is sufficient, whereas in the sequential regime 0 08–0 1 mg is required.

Physiologically the combined method differs from the sequential method inasmuch as in the former both the proliferative and the secretory phases are much shortened and followed by a pseudodecidual phase that completes the cycle When the sequential method is used the pseudodecidual phase is absent, but, instead, the proliferative phase is prolonged and the secretory phase shortened in relation to the course of the normal cycle The sequential method is regarded as an advance in development and signifies less interference with the natural menstrual pattern. The menstrual bleeding proceeds from a more natural mucous membrane which explains why as a rule it is not so greatly reduced as in the combined method.

Clinical trials with the sequential method

Goldzieher (1964) has reported on over 15 000 well observed cycles with the sequential method 15+5 and has used a prepara-

Table I. Side Effects During 483 Cycles i 38 Women

	Before Treatment	Cycle No. 1	2	3	4	5
	No. of Patients	No. of Patients	No. of Patients	No. of Patients	No. of Patients	No. of Patients
Spotting		8 (21 %)	5 (13 %)	4 (11 %)	2 (5 %)	—
Break-through bleeding		—	—	—	—	—
Nausea		5 (13 %)	1 (3 %)	—	—	—
Vomiting		2 (5 %)	1 (3 %)	1 (3 %)		
Painful breasts	2 (5 %)	4 (11 %)	3 (8 %)	2 (5 %)	1 (3 %)	1 (3 %)
Premenstrual tension	9 (24 %)	Symptom decreased				
Headache	7 (18 %)	7 (18 %)	1 (3 %)	—	—	—
Dys-menorrhea	10 (26 %)	Symptom decreased				

see text

Table II. *Amount of Bleeding*

	After 1 7 Cycles No. of Patients	After more than 7 Cycles No. of Patients
Amount of bleeding unchanged	17 (45 %)	17 (45 %)
Amount of bleeding increased	8 (21 %)	—
Amount of bleeding decreased	13 (34 %)	21 (55 %)

ment cycle there was an improvement in premenstrual tension in all 9 patients. After a few more months some of them became entirely free from symptoms. Painful breasts were reported by four patients during the first cycle. Continued treatment with Ovisec resulted in the mitigation of these complaints.

Dysmenorrhea and headache

Eleven patients had been troubled with dysmenorrhea prior to treatment. There was distinct improvement in all of them and in

Material

38 women between the ages of 19 and 43 mean 28 were subjected to trials with Ovisec during a total of 483 cycles. Of the 38 patients 28 were parous Seven patients took tablets for 16 cycles, 6 for 15 cycles and those most recently treated took them for 8 cycles. The patients had requested oral contraceptives at the time of consultation. At their first visit a routine gynaecological examination was established and a general case-report was made. In addition to the gynaecological examination, the breasts were palpated, transaminase values (GOT and GPT) were determined, a cervical smear test was made and weight and Hb were recorded. Each patient was given four packages and requested to note on special control cards any deviations from normal for example spotting breakthrough bleeding, breast tension head ache etc. A separate card was used for each cycle The patients were asked to come for a check before the fourth package was finished.

Results

Side effects were few and of a mild character Tables I and II.

Spotting and break-through bleeding

Spotting had occurred in a number of patients prior to treatment after treatment its frequency decreased and by the 5th cycle no spotting occurred No patient reported marked break through bleeding (BTB)

Nausea and vomiting

Two patients vomited once or twice One of them had had the same trouble before treatment but there was no vomiting after the 3rd cycle Nausea ceased after two cycles.

Premenstrual complaints

Premenstrual tension and painful breasts occurred in 9 and 2 patients respectively before treatment Even after the first treat

Table I. *Side Effects During 483 Cycles in 38 Women*

	Before Treatment	Cycle No. 1	2	3	4	5
	No of Patients	No. of Patients	No. of Patients	No. of Patients	No. of Patients	No. of Patients
Spotting		8 (21 %)	5 (13 %)	4 (11 %)	2 (5 %)	—
Break-through bleeding		—	—	—	—	—
Nausea		5 (13 %)	1 (3 %)	—	—	—
Vomiting		2 (5 %)	1 (3 %)	1 (3 %)		
Painful breasts	2 (5 %)	4 (11 %)	3 (8 %)	2 (5 %)	1 (3 %)	1 (3 %)
Premenstrual tension	9 (24 %)	Symptom decreased				
Headache	7 (18 %)	7 (18 %)	1 (3 %)	—	—	—
Dys-menorrhea	10 (26)	Symptom decreased[1]				

[1] see text

Table II. *Amount of Bleeding*

	After 1 7 Cycles No of Patients	After more than 7 Cycles No. of Patients
Amount of bleeding unchanged	17 (45 %)	17 (45 %)
Amount of bleeding increased	8 (21 %)	—
Amount of bleeding decreased	13 (34 %)	21 (55 %)

ment cycle there was an improvement in premenstrual tension in all 9 patients. After a few more months some of them became entirely free from symptoms. Painful breasts were reported by four patients during the first cycle. Continued treatment with Ovisec resulted in the mitigation of these complaints.

Dysmenorrhea and headache

Eleven patients had been troubled with dysmenorrhea prior to treatment. There was distinct improvement in all of them and in

some instances they became *entirely free from their symptoms*, with the exception of one patient in whom the complaint still persisted in the 8th cycle. Headache prior to treatment was reported by 7 patients: this was mitigated and disappeared after 2 cycles. These headaches were of a mild character and occurred once or twice a month. Two patients suffered from migraine; there was improvement in one case.

Effect on bleeding (Table II)

During treatment the menstruation was normal as regards both cycle and duration. The cycle approximated more closely to 4 weeks. To begin with, bleeding was more abundant in 8 patients, but after 4–7 cycles the amount decreased and was somewhat less than normal. In 13 patients the amount of bleeding was *reduced at once*, whereas in 17 it *remained unchanged*. Cycle control was remarkably good.

Weight

The values given below indicate the changes in weight.

No change in weight	50 % = 19 patients
Increase in weight	24 % = 9 patients
Decrease in weight	26 % = 10 patients

In combined treatment increase in weight is more usual than decrease. In this trial with Ovisec, for the most part, weight remained unchanged or decreased.

Depression

It is well known that caution and restraint should be exercised when giving oral contraceptive agents to women who have either had or shown signs of episodes of depression. This series included 4 patients who had *previously suffered from depression* of from some complaint of a similar nature, but who insisted on trying an oral contraceptive. One of them had previously used Conluten, but she and the other three patients in this group discontinued treatment after varying periods (some months—one year) on account of *increased irritability*.

Table III. *Effect on Sexual Intercourse*

	Increased No. of Patients	Decreased No of Patients	Unchanged No. of Patients
Libido	25 (66 %)	3 (8 %)	10 (26 %)
Orgasm	17 (45 %)	1 (3 %)	20 (53 %)
Frequency of coitus	14 (37 %)	1 (3 %)	23 (61 %)

Sexual Intercourse

Ovisec had a favourable effect on sexual intercourse Table III. All but one of the patients stated spontaneously that the tablets were good, very good or excellent.

Discussion

Trials with different sequential preparations have been reported by several authors (*Tyler* 1966 *Cowan* 1967 and *McBride* 1966) Throughout the sequential method has been regarded very favourably by the patients. *McBride* who made trials with Ovisec, found that of 386 women who had previously used combined tablets all with the exception of two preferred Ovisec. It has been questioned whether the sequential method is as safe as the combined method, since in the former only oestrogenic hormone is administered during the first part of the cycle *McBride* treated 4 0 women for 3462 cycles without a single pregnancy occurring. In the present series one pregnancy occurred despite the fact that the patient, according to her statement, had taken the tablets as instructed She had previously been delivered 3 times and had had 2 miscarriages Prior to the Ovisec treatment she had used Lyndiol mite for 9 months, but had discontinued on account of itching At the time of the expected ovulation—*in the* 13th treatment cycle with Ovisec—she had had enteritis with mild diarrhoea. Pregnancy now in the fifth month, was accepted with gladness, as she had intended to have another pregnancy in the near future

The side effects reported in this investigation were throughout, most troublesome during the first cycles, and thereafter rapidly

disappeared. This shows good agreement with the results of *McBride's* investigation into Ovisec.

At the follow-up the gynaecological condition of the patients was found to be normal All the transaminase values and the Hb estimations were normal At the preliminary examination 3 patients had had atypical cytologic tests but subsequent tests were normal No case of jaundice or trombosis was observed.

In treatment by the sequential method it is important that the patients take the tablets in the proper order The design of the Ovisec package the enclosed directions and the different colours of the tablets reduce the risk of the tablets being used in the wrong way Contrary to other oral contraceptive agents a tablet L taken daily since seven placebo tablets are taken during the period previously exempt from tablets.

SUMMARY

A sequential preparation (under the name of Ovisec) was tested during 483 cycles on 38 women. The treatment caused a few mild side effects in the first cycles. 11 patients suffered from dysmenorrhea prior to treatment, and these all became free from symptoms There was improvement in respect of premenstrual trouble cycle control and sexual intercourse Depression was the cause of 4 patients discontinuing treatment One pregnancy occurred

REFERENCES

Cowan L E Canad Med Ass J *96* 1208 1967
Goldzieher J W Med. Clin N Am. *48* 529 1964
McBride W G Med J Austral. *1* 172, 1966
Mears E B 1 Council 211 1965
– Proc. Roy Soc Med *57* 204 1964
Pincus G Rock J Garcia C R Rice-wray M Paniagua I and *Rodrigues, L,* Am. J Obstet Gynaecol *75* 1333 1958
Tyler E. T Matsner E M Gotlib M Lewin M Tacker J S and *Parrott F M.,* J A. M. A *197* 943 1966

Received on Dec. 9 1967

Fjällbrant, Bo, and Obrant Ola, Acta obst et gynec scandinav 47 451, 1968

From the Institute of Medical Microbiology Department of Bacteriology (Professor O Ouchterlony) University of Göteborg, the Department of Obstetrics and Gynaecology II (Professor P Bergman) and the Department of Urology Sahlgrenska sjukhuset Göteborg, Sweden

CLINICAL AND SEMINAL FINDINGS IN MEN WITH SPERM ANTIBODIES

BY

BO FJÄLLBRANT AND OLA OBRANT

Introduction

Most of the reports on men with sperm antibodies have concerned patients found in special urological or infertility clienteles. Hence investigators have often suggested a relationship between the presence of sperm antibodies in the blood and genital or seminal defects. Of *Wilson*'s (1954 1956) 3 men with sperm agglutinins found in a sterility clientele one had oligozoospermia and two had normal sperm counts. *Rümke*'s (1954) first two cases with sperm antibodies were found in a group of 80 males with azoo- or extreme oligozoospermia About one-third of the 67 patients with sperm agglutinins reported by *Rümke and Hellinga* (1959) had azoospermia These authors found a statistically significantly higher incidence of occlusion of the efferent ducts in connexion with normal spermatogenesis in these patients with sperm agglutinins in comparison to the incidence in a group of azoospermic patients without sperm agglutinins. They concluded that spermatostasis resulting from an occlusion of the vas deferens or the epididymal duct might lead to the formation of sperm antibodies. In another third of the same material there was complete or partial autoagglutination of the sperm the morphology of the spermatozoa was always normal (*Rümke* 1965) A relationship between obstructive azoospermia and sperm antibodies was also proposed by *Phadke and Padukone* (1964) who in

disappeared This shows good agreement with the results of *McBride s* investigation into Ovisec.

At the follow-up the gynaecological condition of the patients was found to be normal All the transaminase values and the Hb estimations were normal. At the preliminary examination 3 patients had had atypical cytologic tests, but subsequent tests were normal No case of jaundice or trombosis was observed.

In treatment by the sequential method it is important that the patients take the tablets in the proper order The design of the Ovisec package the enclosed directions and the different colours of the tablets reduce the risk of the tablets being used in the wrong way Contrary to other oral contraceptive agents a tablet i taken daily since seven placebo tablets are taken during the period previously exempt from tablets.

SUMMARY

A sequential preparation (under the name of Ovisec) was tested during 483 cycles on 38 women The treatment caused a few mild side effects in the first cycles. 11 patients suffered from dys menorrhea prior to treatment and these all became free from symptoms. There was improvement in respect of premenstrual trouble cycle control and sexual intercourse Depression was the cause of 4 patients discontinuing treatment One pregnancy occurred

REFERENCES

Cowan L E Canad Med A s J *96* 1208 1967
Goldzieher J W Med Clin. N Am. *48* 529 1964
McBride W G Med J Austral *1* 172 1966
Mears E. Biol Coun 1 ?11 1965
– Proc. Roy Soc Med 57 204 1964
Pincus G Rock J G cia C-R Rice-wray M Paniagua I and *Rodrigues I* Am. J Obstet Gynaecol. *75* 1333 1958
Tyler E T Matsner E M Gotlib M. Lewin M Tacker J S. and *Parrott F M* J A. M A *197* 943 1966

Received on Dec. 9 1967

described in an earlier paper (*Fjällbrant* 1968 a) ranged between 1 4–1 8192. The sperm immobilizing activity of their blood, *i.e* the concentration of sperm immobilizing antibodies was determined as described in an earlier paper (*Fjällbrant* 1968 b) The employed measure of immobilizing activity of the serum was the time required to reduce the percentage of motile donor spermatozoa from about 70 to 10 in the presence of complement this time ranged between 1 hour 10 min. and 60 hours.

The men varied in age from 23 to 44 years (mean 32 years) Of the 43 men 29 lived in sterile and 14 in fertile marriages. The duration of sterility varied between 2 and 12 years. In the fertile marriages 5 of the wives were pregnant, and the other 9 had 1 to 4 children. These 9 wives had been delivered within the 13 months prior to the investigation of their husbands. In these cases the Rh genotype as well as the ABO MN Kell and Duffy blood groups were determined in the husbands, wives and children In no case did the results challenge the paternity of the husband.

Case histories were obtained during interviews at the time of the urological examinations. One man had coitus once a month, all the others one or several times a week. One man had insufficient erection during the period of the sterility investigation the others had no sexual disturbances. Ten men had been treated for or had had signs and symptoms of disease of the urogenital organs, namely gonorrhoea (2) mumps orchitis (2) epididymitis (3 or possibly 4) and micturition disturbances (7) In no case had the diagnosis of prostatovesiculitis been established earlier Four males had suffered scrotal trauma with oedema haematoma or other visible signs. In 2 cases a testicular biopsy had been performed earlier Two patients had been submitted to operation for inguinal hernia

In semen samples from 34 of the men the mucus penetration ability of the spermatozoa was investigated with the method described by Kremer (1965) slightly modified as described in an earlier paper (*Fjällbrant* 1969 b) One end of a capillary tube filled with ovulatory cervical mucus was immersed into the semen, and the extent of the sperm penetration was measured after 3 hours The extent was ≤5 mm in 12 and >5 mm in 22 cases.

blood samples from 50 azoospermic men with proven obstruction of the vas deferens found sperm agglutinins in 13 In 6 cases out of 25 in whom the obstruction had been relieved agglutinins persisted for years. *Cruickshank and Stuart Smith* (1959) found 2 men with sperm agglutinins among 6 cases with granulomatous orchitis and 8 cases of epididymitis and/or orchitis. A relationship between sperm antibodies and genital inflammatory conditions was suggested also by *Bandhauer* (1963) Of 75 patients with genital infections he found sperm agglutinins in 9 *Fjällbrant* (1965) reported 11 cases with sperm antibodies found among sterility patients. Ten of them had normal sperm counts and only one slight oligozoospermia There were no apparent genital defects In a male urological clientele comprising 498 patients *Bandhauer* (1966) found 29 patients who showed serological agglutinin positivity (defined as a titre ≥ 1 32 with Kibrick s method) The incidence of sperm agglutinin pisitivity was rather low in cases with some kind of prostatovesiculitis (4 107) but *considerably higher in males with epididymitis (16 101)* Ejaculates from 25 of the sperm agglutinin positive males were examined In nearly half of them the sperm density was ≤ 40 millions/ml In 4 there was azoospermia and in more than half of the samples the percentage of motile spermatozoa was ≤ 55

The aim of the present investigation was to analyse the clinical and seminal findings in men with sperm antibodies in their blood and relate these findings to the sperm antibody level the mucus penetration ability of the spermatozoa and fertility

Material and Methods

The investigation concerned 43 men with sperm antibodies in the blood. The presence of sperm agglutinins in the blood of 36 of them was discovered during an investigation of a large group of male partners of female patients at the Department of Obstetrics and Gynaecology II living in sterile or fertile marriages (*Fjällbrant* 1968 a) The other 7 men with sperm agglutinins were found in the sterility clientele of the department. The sperm agglutinin titres determined with the macroscopic, direct sperm agglutination test of *Kibrick et al* (1952) slightly modified as

ant, who had investigated semen samples for many years. The ejaculates were examined for the presence of mucus clumps and abnormal viscosity Semen volume was measured in a tube graduated in tenths of millilitres. Sperm density was determined with a Buerker chamber The percentage of motile spermatozoa was counted microscopically on a slide. The degree of motility was estimated in the same manner and registered numerically (1 = motility *in loco* without forward movement, 2–4 = slow rapid, very rapid forward movement) The type of sperm agglutination was noted and the agglutination degree registered as 0 = none, 1–3 = small medium, large aggregates. The sperm morphology was studied in a smear stained according to Moench (1930) *i.e* Ziehl-Neelsen's carbolfuchsin eosin counterstaining with methylene blue The heads were classified according to Hammen (1944) heads with vacuoles were, however noted as normal. Examination for the presence of cells other than spermatozoa was made in a smear stained with the May-Grünwald-Giemsa technique For each type of cell except the mature spermatozoon the finding of one or more cells per high power field (1000×) was classified as abnormal. Fructose concentration was determined with the technique described by Mann (1964) Acid phosphatase activity was determined with a King Armstrong method modified for the Auto-Analyser (pH 4.9 temp 37 C substrate disodiumphenyl phosphate) a value ≤ 120 000 U/100 ml was classified as abnormal.

Statistical calculations

The comparison between distributions was made with X^2 tests. All tests were carried out at a 5 per cent level of significance. A difference was called significant when $p < 0.05$ highly significant when $p < 0.01$ and almost significant when $p < 0.1$

Results

Clinical findings

In 21 men with sperm antibodies no pathological conditions were observed. In the remaining 22 the following pathological conditions were encountered

A group of 40 men without sperm agglutinins were selected from the clientele of the Department of Surgery They were examined only for the presence of prostatovesiculitis besides the examination of their blood for sperm agglutinins. The age of this group was 18–45 years mean 32 years. Their surgical disease was assumed not to interfere with the state of their genital organs (e g leg contusions concussions)

Clinical Investigation

Palpation of the testis epididymis and the vas deferens was performed with patients in the upright position palpation of the prostate and seminal vesicles was done with the patient standing leaning forward with the elbows resting on a support. After palpation of the male adnexal glands they were compressed and stripped. If secretion was obtained which occurred in ¾ of the cases this was collected on a glass slide and analysed immediately by microscopy without staining A urine sample for analysis of sediment was taken in those subjects from whom no secretion was obtained.

Any change in the consistency of the male adnexal glands was considered to indicate prostatovesiculitis but this diagnosis was never set on the palpation findings alone For this diagnosis it was required that the secretion show at least 20 leucocytes per microscopic field (600×) or that the urine collected after compression and stripping show at least 10 leucocytes per field.

Seminal Investigation

Only one semen sample was requested from the men who were fathers in the majority of the cases in the sterility clientele several samples were investigated. There were differences between the samples from the same patient, although generally minor ones The results obtained with the semen sample delivered at the time the blood was taken for analysis of sperm antibody content were employed for the interrelation studies

The semen samples were investigated 1–2 hours after ejaculation The investigation was performed according to the routine of the laboratory All the samples were examined by one assist

Table I *Means and Ranges for Some Semen Properties in 43 Men with Sperm Antibodies*

Semen Property	Mean	Range
Volume	3.5 ml	0.3–8.4 ml
Density	143 millions/ml	<1 544 millions/ml
Abnormal heads	29 %	7–81 %
Motile	50 %	6–82 %
Motility degree	2.7	1–4
Tail to-tail agglutination degree	1.2	0–3
Fructose	416 mg %	12–690 mg %
Acid phosphatase	441,000 U/100 ml	80,000–1,000,000 U/100 ml

Table II *The Distribution of the Ejaculates with Regard to Semen Properties in 43 Men with Sperm Antibodies*

Volume (ml)	>2.0	2.0–1.0	<1.0	
Number	33	9	1	
Density (millions/ml)	>60	41–60	21–40	≤20
Number	31	2	7	3
Abnormal heads (%)	<40	40–49	50–59	≥60
Number	33	4	4	1
Motile (%)	>70	51 70	31 50	≤30
Number	9	13	10	10
Motility degree	4	3	2	1
Number	10	17	9	6
Agglutination degree	0	1	2	3
Number	12	13	13	4

also for motility degree Most of the samples showed some degree of tail to-tail agglutination

It may be noted that there was no case of azoospermia and only one case of extreme oligospermia for this case the percent age of motile spermatozoa the motility degree the percentage of abnormal heads and the agglutination degree could not be measured It is also noteworthy that head-to-head agglutination

prostatovesiculitis	9
varicocele	4
hard noduli in epididymis	1
suspect unilateral spermatocele	1
prostatovesiculitis + varicocele	4
prostatovesiculitis + hard noduli in epididymis	1
prostatovesiculitis + unilateral testis atrophy	1
prostatovesiculitis + varicocele + hard noduli in epididymis	1

Thus prostatovesiculitis was found in 16 men (37 per cent) In one case the prostatovesiculitis proved to be tuberculous. Varicocele in all cases left side was found in 9 patients (21 per cent)

In the 40 men without sperm agglutinins examined for the presence of prostatovesiculitis this condition was found in 6 (15 per cent) The difference between this group and the sperm agglutinin positive group in regard to the incidence of prostatovesiculitis (6 40 and 16 43 respectively) was statistically significant ($X^2 = 5.25$ d f = 1 $p < 0.05$)

Seminal findings

Table I shows the means and ranges of the most important semen properties in men with sperm antibodies It can be seen that the ranges were very wide in fact the samples varied from almost normal to highly pathological ones. The means for volume sperm density fructose and acid phosphatase were high and the mean for abnormal heads low The means for percentage of motile spermatozoa and motility degree were however low and the mean for the degree of tail to-tail agglutination was 1 2. The trend toward pathological values for these last three properties in comparison to the other semen properties is more readily seen in Table II which shows the distribution of the ejaculates with regard to semen properties *The majority of the samples* had high values for volume (> 2.0 ml) and density (> 60 millions/ml) and low for abnormal heads (< 40 %) In regard to motile spermatozoa however the majority had low (31–70 %) or very low (≤ 30 %) values and the majority had low values (≤ 3)

Table III. Cases *with and without* Prostatovesiculitis Distributed *with Regard* to Semen Properties

Semen Property		Without Prostato-vesiculitis		With Prostato-vesiculitis	
Volume (ml)	> 2.0	21	(78 %)	12	(75 %)
	≤ 2.0	6	(22 %)	4	(25 %)
Density (millions/ml)	> 60	22	(81 %)	9	(56 %)
	≤ 60	5	(19 %)	7	(44 %)
Abnormal heads (%)	< 40	21	(81 %)	12	(75 %)
	≥ 40	5	(19 %)	4	(25 %)
Motile (%)	> 50	14	(54 %)	8	(50 %)
	≤ 50	12	(46 %)	8	(50 %)
Motility degree	3 or 4	18	(69 %)	9	(56 %)
	1 or 2	8	(31 %)	7	(44 %)
Agglutination degree	0 or 1	15	(58 %)	10	(62 %)
	2 or 3	11	(42 %)	6	(38 %)

distribution of men with and men without prostatovesiculitis as well as men with and men without varicocele in the fertile and sterile groups was rather equal.

Seminal findings and clinical findings

In Table III cases with and cases without prostatovesiculitis are distributed according to semen properties. Only as regards density was there a tendency to a higher incidence of pathological values in the prostatovesiculitis group ($X^2 = 3.18$, d.f. = 1, $p < 0.1$).

Sperm antibody level and seminal findings

In Table IV cases with low (≤ 1: 32) and high (≥ 1: 64) sperm agglutinin titres are distributed with regard to semen properties. The general trend was a higher incidence of pathological values in the group with high agglutinin titres. Statistical analysis of the figures, however, showed that motility degree was the only property that showed a significant difference between the groups

or mixed agglutination was never encountered. In all the samples with apparent agglutination thus was of the tail-to-tail type.

The viscosity was normal in most of the ejaculates in 8 samples there were minor mucus clumps and one ejaculate showed an abnormally low viscosity An abnormally high number of leucocytes was found in 6 ejaculates of erythrocytes in 4 of macrophages in 1 of epithelial cells in 5 and of bacteria in 3 the number of testicular cells was normal in all the samples. The low fructose values noted were combined with a high sperm density and a high motility degree i e they could be explained by a high fructose consumption There were only 4 low phosphatase values and these were found in patients with acute prostatovesiculitis.

In all of the 10 ejaculates with motility degree 4 the sperm density was high (> 60 millions/ml) Of 27 ejaculates with motility degree 3 or 4 only 3 showed a low (≤ 60 millions/ml) sperm density but of 15 samples with motility degree 1 or 2 there were 8 with low motility This difference with regard to sperm density between ejaculates with motility degree 3 or 4 and 1 or 2 was statistically highly significant ($\chi^2 = 8.89$ d.f. $= 1$ $p < 0.01$)

Clinical findings and sperm agglutinin titre

Of the 16 patients with prostatovesiculitis 7 had low and 9 high agglutinin titres of 27 patients without prostatovesiculitis 14 had low and 13 high agglutinin titres Of 9 patients with varicocele 5 had low and 4 high agglutinin titres of 34 men without varicocele 16 had low and 18 high agglutinin titres. Thus men with and men without prostatovesiculitis as well as men with and men without varicocele were nearly equally distributed in the groups with low and high sperm agglutinin titres.

Clinical findings and fertility

Of the 16 patients with prostatovesiculitis 6 belonged to the fertile and 10 to the sterile group for 27 men without prostatovesiculitis the corresponding figures were 8 and 19 Of the 9 patients with varicocele 2 were fertile and 7 sterile for 34 patients without varicocele the corresponding figures were 12 and 22. Thus the

Table VI. *The Distribution of the Cases in Regard to Sperm Motility Degree in the Ejaculate in Relation to the Sperm Immobilizing Activity of the Serum*

Immobilizing Activity	Motility Degree				Total
	1	2	3	4	
<3 h	2	7	2		11
3–<6 h	4	1	5	2	12
6–<12 h			4	2	6
12 h		1	6	6	13
Total	6	9	17	10	42

significant, r = 0.42. Table VI shows that the statistically significant negative correlation between immobilizing activity of the serum and motility degree in the ejaculate (r = −0.57) was still higher than the aforementioned correlation.

Sperm agglutinin titre and fertility

In the fertile group there were 11 men with low sperm agglutinin titres and only 3 with high titres. In the sterile group there were 10 with low and 19 with high sperm agglutinin titres. The difference between the groups with regard to sperm agglutinin titre was highly significant ($X^2 = 7.35$ d.f. = 1 $p < 0.01$)

Seminal findings and fertility

In Table VII the fertile and sterile groups are distributed with regard to semen properties. There were greater incidences of pathological values in the sterile group. The difference was very great and statistically highly significant for motility degree ($X^2 = 11.67$ d.f. = 1 $p < 0.01$) and smaller though statistically significant for density ($X^2 = 4.45$ d.f. = 1 $p < 0.05$). The difference between the groups was statistically almost significant for agglutination degree ($X^2 = 3.16$ d.f. = 1 $p < 0.1$) and for volume ($X^2 = 3.02$, d.f. = 1 $p < 0.1$)

Seminal findings and mucus penetration ability

In Table VIII the penetration ability of the spermatozoa in the 34 semen samples examined in this respect is related to the pro-

Table IV *Cases with Low (≤1 32) and High (≥1 64) Sperm Agglutinin titre Distributed with Regard to Semen Properties*

Semen Property		Low Titre		High Titre	
Volume (ml)	>2.0	17	(81 %)	16	(73 %)
	≤2.0	4	(19 %)	6	(27 %)
Density (millions/ml)	>60	17	(81 %)	14	(64 %)
	≤60	4	(19 %)	8	(36 %)
Abnormal heads (%)	<40	18	(90 %)	15	(68 %)
	≥40	2	(10 %)	7	(32 %)
Motile (%)	>50	12	(60 %)	10	(45 %)
	≤50	8	(40 %)	12	(55 %)
Motility degree	3 or 4	18	(90 %)	9	(41 %)
	1 or 2	2	(10 %)	13	(59 %)
Agglutination degree	0 or 1	15	(75 %)	10	(45 %)
	2 or 3	5	(25 %)	12	(55 %)

Table V *The Distribution of the Cases in Regard to Sperm Agglutination Degree in the Ejaculate in Relation to the Sperm Agglutinin Titre of the Serum*

Agglutinin Titre (reciprocal)	Agglutination Degree				Total
	0	1	2	3	
4096–8192	1		1		2
1024–2048		1	3	2	6
256–512		2	1	1	4
64–128	2	4	3	1	10
16–32	5	4	4		13
4–8	4	2	1		7
Total	12	13	13	4	42

with low and high agglutinin titres This difference was very great and statistically highly significant ($X^2 = 11.00$ d.f = 1 $p < 0.01$) For the agglutination degree the difference between the groups was almost significant ($X^2 = 3.80$ d.f = 1 $p < 0.1$)

Table V confirms the tendency to higher agglutination degree with increasing agglutinin titre the correlation was statistically

Table VI. The Distribution of the Cases in Regard to Sperm Motility Degree in the Ejaculate in Relation to the Sperm Immobilizing Activity of the Serum

Immobilizing Activity	Motility Degree				Total
	1		3	4	
< 3 h	2	7	2		11
3–< 6 h	4	1	5	2	12
6–< 12 h			4	2	6
≥ 12 h		1	6	6	13
Total	6	9	17	10	42

significant, r = 0.42. Table VI shows that the statistically significant negative correlation between immobilizing activity of the serum and motility degree in the ejaculate (r = −0.57) was still higher than the aforementioned correlation.

Sperm agglutinin titre and fertility

In the fertile group there were 11 men with low sperm agglutinin titres and only 3 with high titres. In the sterile group there were 10 with low and 19 with high sperm agglutinin titres. The difference between the groups with regard to sperm agglutinin titre was highly significant ($X^2 = 7.35$ d.f. = 1 $p < 0.01$)

Seminal findings and fertility

In Table VII the fertile and sterile groups are distributed with regard to semen properties. There were greater incidences of pathological values in the sterile group. The difference was very great and statistically highly significant for motility degree ($X^2 = 11.67$ d.f. = 1 $p < 0.01$) and smaller though statistically significant for density ($X^2 = 4.45$ d.f. = 1 $p < 0.05$). The difference between the groups was statistically almost significant for agglutination degree ($X^2 = 3.16$ d.f. = 1 $p < 0.1$) and for volume ($X^2 = 3.02$, d.f. = 1 $p < 0.1$)

Seminal findings and mucus penetration ability

In Table VIII the penetration ability of the spermatozoa in the 34 semen samples examined in th

Table VII *The Fertile and Sterile Groups Distributed with Regard to Semen Properties*

Semen Property		Fertile		Sterile	
Volume	> 2.0	13	(93 %)	20	(69 %)
(ml)	≤ 2.0	1	(7 %)	9	(31 %)
Density	> 60	13	(93 %)	18	(62 %)
(millions/ml)	≤ 60	1	(7 %)	11	(38 %)
Abnormal heads	< 40	12	(86 %)	21	(75 %)
(%)	≥ 40	2	(14 %)	7	(25 %)
Motile	> 50	9	(64 %)	13	(46 %)
(%)	≤ 50	5	(36 %)	15	(54 %)
Motility	3 or 4	14	(100 %)	13	(46 %)
degree	1 or 2	0	(0 %)	15	(54 %)
Agglutination	0 or 1	11	(79 %)	14	(50 %)
degree	2 or 3	3	(21 %)	14	(50 %)

Table VIII. *Relations Between Semen Properties and Mucus Penetration Ability of Spermatozoa in 34 Semen Samples*

Semen Property		Penetration ≤ 5 mm		Penetration > 5 mm	
Volume	> 2.0	10	(83 %)	16	(82 %)
(ml)	≤ 2.0	2	(17 %)	4	(18 %)
Density	> 60	5	(42 %)	21	(95 %)
(millions/ml)	≤ 60	7	(58 %)	1	(5 %)
Abnormal heads	< 40	8	(67 %)	21	(95 %)
(%)	≥ 40	4	(33 %)	1	(5 %)
Motile	> 50	4	(33 %)	17	(77 %)
(%)	≤ 50	8	(67 %)	5	(23 %)
Motility	3 or 4	4	(33 %)	21	(95 %)
degree	1 or 2	8	(67 %)	1	(5 %)
Agglutination	0 or 1	6	(50 %)	17	(77 %)
degree	2 or 3	6	(50 %)	5	(23 %)

perties of the semen There was a striking interrelation between low penetration rate and pathological values for most of the

semen properties. The difference between the groups with penetration ≤5 mm and >5 mm was greatest with regard to motility degree 21 samples of 25 with degree 3 or 4 showed a penetration >5 mm, and 8 of 9 samples with degree 1 or 2 showed a penetration ≤5 mm. This difference was statistically highly significant ($X^2 = 15.40$ d.f = 1 $p < 0.01$) The difference between the groups was highly significant also for density ($X^2 = 12.48$, d.f = 1 $p < 0.01$) less but significant for the percentage of motile spermatozoa ($X^2 = 6.35$ d.f = 1 $p < 0.05$) and of abnormal heads ($X^2 = 5.13$ d.f = 1 $p < 0.05$)

Discussion and Conclusions

The case histories did not show any discordant features. The men s sexual life was normal. Urogenital disease had occurred in less than one-fourth of the men. Also the incidence of urogenital trauma and operation was small.

The results of the clinical and seminal investigations, however are noteworthy in some respects. The incidence of varicocele was 9 43 or 21 per cent, which agrees with the incidence of varicocele in young adults of 15–20 per cent stated by *Lane Roberts et al* (1948) There was no relationship between the presence of varicocele on the one hand and the sperm agglutinin titre or fertility on the other The incidence of prostatovesiculitis was 16 43 or 37 per cent. The difficulties in comparing the incidences of prostatovesiculitis found by different investigators are well known In those earlier investigations which include some kind of control group the incidence of prostatovesiculitis of the pathological group is often twice or more as great as that of the controls (*Domeij et al* 1958 *Mason et al.* 19_8) The present investigation shows the same pattern as the incidence of prostatovesiculitis of the control group was 6 40 or 15 per cent. No relationship was found between the presence of prostatovesiculitis and the level of sperm antibodies in the blood, nor between prostatovesiculitis and semen properties or fertility Thus the only remarkable clinical finding is an unexpected high incidence of prostatovesiculitis *Bandhauer* (1966) found a rather low coincidence of sperm agglutinin positivity and prostatovesiculitis (without giving his criteria for the diagnosis) *Rümke* and *Hellinga*

(1959) and *Rümke* (1965) did not mention prostatovesiculitis. The high incidence of prostatovesiculitis in men with sperm antibodies found in the present investigation however suggests a relationship between prostatovesiculitis and sperm antibodies which deserves further investigation

In comparison to the findings of other investigators particularly *Rümke* and *Hellinga* the most striking difference is the complete absence in the present investigation of cases with azoospermia and the low incidence of oligozoospermia The high sperm density the low percentage of abnormal spermatozoa the normal number of testicular cells and the finding of normal testes at the clinical examination favour the assumption of intact spermatogenesis in the majority of the males. For these reasons no testicular biopsies were performed.

The most striking feature of the semen samples is the deviation in percentage of motile spermatozoa motility degree and agglutination degree in relation to the other semen properties in the majority of the ejaculates (Tables I and II) Table III shows that the values for these deviating properties were not related to the presence of prostatovesiculitis. The existence of a strong interrelation between the sperm antibody level of the serum and the sperm motility degree in the ejaculate is suggested by the finding of a correlation between the sperm immobilizing activity of the serum and sperm motility and also by the great and statistically highly significant difference with regard to sperm motility between cases with low and high sperm agglutinin titres. As regards the agglutination degree in the ejaculate the difference between cases with low and high agglutinin titres is not so great, but there is in fact a statistically significant correlation between sperm agglutination degree and the agglutinin titre The percentage of motile spermatozoa shows no clear relationship to the sperm antibody level of the serum The deviation of the values for sperm motility degree and sperm agglutination degree of the ejaculates however seems explainable through the action of the sperm immobilizing and sperm agglutinating antibodies present in these men

The coefficient of correlation between motility degree and the sperm immobilizing activity of the serum was -0.57 i e only

moderately high. An explanation of this might be the interrelation found between motility degree and sperm density. The existence of such an interrelation is suggested by the facts that all cases with motility degree 4 had a high sperm density (>60 millions/ml) and that the difference between ejaculates with motility degree 3 or 4 and ejaculates with motility degree 1 or 2 as regards the incidence of high sperm density was great and highly significant. If the sperm density is high, the sperm antibodies available in the seminal plasma presumably are distributed among more spermatozoa than in ejaculates with low sperm density and thus each spermatozoon may be less affected by sperm antibodies.

The correlation between the sperm agglutinin titre in the serum and the sperm agglutination degree in the ejaculate was not as high as that between immobilizing activity and motility (0.42 and −0.57 respectively). Probably the explanation for this difference between the correlations is that the spermatozoa must collide if agglutination is to occur: a high concentration of immobilizing sperm antibodies decreases the motility of the spermatozoa which consequently do not agglutinate despite a high agglutinin titre. There were 3 cases with high sperm agglutinin titres in which there was no agglutination in the ejaculates. If screening for subjects with sperm antibodies had been performed only on the basis of the presence or absence of agglutination in the ejaculates these 3 cases would not have been revealed.

The existence of an interrelation between high sperm antibody levels in the blood and sterility has been stated earlier (Fjällbrant 1968 a and b). In the present investigation this interrelation is expressed through the great and highly significant difference between the fertile and sterile groups with regard to the sperm agglutinin level. A similar discrepancy between the fertile and sterile groups was also found with regard to sperm motility degree. The most remarkable finding in table VII is the fact that no semen sample with motility degree 1 or 2 was found in the fertile group. As this group should be less influenced by female infertility factors than the sterile group, this finding makes the strong relationship between sperm motility degree and fertility still more relevant.

Thus relationships are found between high sperm antibody levels in the blood and decreased motility of the spermatozoa in the ejaculate between high sperm antibody levels and sterility and between decreased sperm motility and sterility In an earlier investigation (*Fjällbrant* 1968 b) made largely on the same material relationships were found between high sperm antibody levels in the blood and reduced penetration of cervical mucus by spermatozoa between high sperm antibody levels and sterility and between reduced mucus penetration and sterility The findings of the two investigations imply a relationship between decreased sperm motility and reduced penetration. This relationship was also found in the 34 cases examined in this respect (Table VIII)

The results of the two investigations make apparent the existence of an interrelation of high sperm antibody level in the blood – decreased sperm motility in the ejaculate – reduced mucus penetration of spermatozoa – sterility It is proved by the sperm immobilizing activity test that sperm antibodies cause immobilization of spermatozoa The presence of γ-globulins and also sperm agglutinins in the seminal plasma has been observed by several investigators (*Wilson* 1954 *Rumke* and *Hellinga* 1959 *Klopstock et al* 1963 *Eyquem et al* 1966 *Fjällbrant* unpublished) Mucus penetration requires good motility Impaired mucus penetration is a cause of sterility Thus it seems probable that the relationship between sperm antibodies at high levels in blood and sterility may be due to reduced motility and penetration ability of spermatozoa in the ejaculate

SUMMARY

The clinical and seminal findings were analysed in 43 men with sperm antibodies in their blood Prostatovesiculitis was found in 16 men or 37 per cent (a statistically significantly higher incidence than in a sperm agglutinin negative group of 40 men of similar age) Sperm agglutination of the tail to-tail type was found in most of the semen samples and in the majority there was also a low percentage of motile spermatozoa and a low degree of sperm motility without corr sponding deviations of the other semen

properties. There were statistically significant correlations between the immobilizing activity of the serum and the sperm motility degree in the ejaculates and between the sperm agglutinin titre of the serum and the sperm agglutination degree in the semen. An interrelation was found between high sperm antibody level in the blood, decreased sperm motility in the ejaculate and sterility.

It is concluded that the reduced motility and the agglutination of spermatozoa in the ejaculates can be explained by the sperm antibodies present in the investigated males and that the relationship between sperm antibodies at high levels in the blood and sterility might be due to reduced motility and penetration ability of the spermatozoa in the ejaculate. The high incidence of prostatovesiculitis in men with sperm antibodies suggests a causative relationship between the presence of sperm antibodies and prostatovesiculitis which deserves further investigation.

Acknowledgements

Miss Yvonne Steen performed as technical assistant. The English text was revised by Translator C. Wadsworth and the statistical calculations by Lector E. Carlström, the Department of Statistics University of Göteborg. The investigation was supported by grants from the Medical Society of Göteborg and the Faculty of Medicine University of Göteborg.

REFERENCES

Bandhauer K. Untersuchungen über immunbiologische Ursachen der männlichen Sterilität. Klin. Med. 18, 204, 1963.

Bandhauer K. Immunreaktionen bei Fertilitätsstörungen des Mannes. Urol. int. (Basel) 21, 247, 1966.

Cruickshank B. and Stuart-Smith D. A. Orchitis associated with sperm-agglutinating antibodies. Lancet 1, 708, 1959.

Domeij B., Giertz G., Olhagen B. and Romanus R. Genitourinary focus in rheumatic disorder in the male. Acta chir. scand. 115, 1, 1958.

Fréquin A., Jouanne C. et Laudet S. Contribution à l'étude des constituants antigéniques du plasma séminal humain. Ann. Inst. Pasteur 110 suppl. 89, 1966.

Hallbrant B. Immunoagglutination of sperm in cases of sterility. Acta obstet. gynec. scand. 44, 474, 1965.

– Sperm agglutinins in sterile and fertile men. Acta obstet. gynec. scand. 47 xxx 1968 a
– Interrelation between high levels of sperm antibodies reduced penetration of cervical mucus by spermatozoa and sterility in men. Acta obstet. gynec. scand 47 xxx 1968 b
Hammen R. Studies on impaired fertility in man with special reference to the male Munksgaard Copenhagen 1944
Kibrick S *Belding* D L. and *Merrill* B. Methods for the detection of antibodies against mammalian spermatozoa. II. A gelatin agglutination test. Fertil and Steril. 3 430 1952
Klopstock A. *Haas* R. and *Rimon* A. Immunoelectrophoretic analysis of seminal plasma. Fertil and Steril 14 530 1963
Lane-Roberts C. *Sharman* A. *Walker* K. *Wiesner* B. P and *Barton* M. Sterility and impaired fertility Hamish Hamilton Medical Books London, 1948
Mann T The biochemistry of semen and of the male reproductive tract. Methuen London, 1964
Mason R. M *Murray* R. S *Oates* J K. and *Young*, A. C. Prostatitis and ankylosing spondylitis Brit. med. J 5073 748 1958
Moench G L The technic of the detailed study of seminal cytology Amer J Obstet. Gynec. 19 530 1930
Phadke A. M and *Padukone* K. Presence and significance of autoantibodies against spermatozoa in the blood of men with obstructed vas deferens. J Reprod Fertil 7 163 1964
Rümke P The presence of sperm antibodies in the serum of two patients with oligozoospermia Vox Sang (Basel) 4 135 1954
Rümke P and *Hellinga* G Autoantibodies against spermatozoa in sterile men Amer J clin. Path 32 357 1959
Rümke P Autospermagglutinins a cause of infertility in men. Ann. N Y Acad. Sci 124 696 1965
Wilson L Sperm agglutinins in human semen and blood. Proc. Soc. exp. Biol (N Y) 85 652, 1954
Wilson L Sperm agglutination due to autoantibodies A new cause of sterility Fertil. and Steril 7 262 1956

Received on Dec. 7 1967

Nodskov Pedersen, S. Acta obst. et gynec. scandinav. 47 [illegible], 1968

From the Fibiger Laboratory, Kgs. Lyngby (J. Kieler M.D.) Bispebjerg Hospital Dept. K (Aug. Christensen M.D.) and The Radium Centre (H. Johansen) Copenhagen, Denmark

RESPIRATION AND GLYCOLYSIS IN MALIGNANT AND NON-MALIGNANT TISSUE FROM THE HUMAN CERVIX UTERI

BY

S. NODSKOV PEDERSEN

The degree of malignancy of cancer of the human cervix uteri is usually evaluated on the basis of histological findings (*Wentz et al.* 1965) and the clinical stage as defined by international committees in Austria in 1961 and in Argentina in 1964. Although the prognostic value of observations of this type is well established they only give us limited information about how and to what extent the malignant cells deviate from normal cells. Such information would be of great interest not only from a theoretic point of view, but also from a clinical point of view, particularly when the degree of malignancy of early cancers is to be evaluated.

The present work is part of a clinical-experimental investigation which was carried out in order to clarify whether the clinical and pathological classification of cervical carcinomas can be correlated with metabolic and other observations made *in vitro*.

Among the numerous biochemical abnormalities which have been demonstrated in malignant tumours, their high rate of glycolysis first described by Otto Warburg (1930) is so far the only one which can be characterized as typical of most malignant tumours. This has been confirmed by many later authors, but clinically only limited use has been made of this observation. One of the reasons for this is probably due to the difficulties presented by the lack of homogeneity of the tissue. Several authors (*Aisenberg*, 1961; *De Roetth* 1957; *Dickens et al.* 1930) have tried to correct for this by determinations of the so-called carcinoma-

Under the auspices of The Danish Cancer Society

cellularity index which is a measure of the percentage of tumour cells counted in histological slides

The metabolism of cervical carcinomas in women has previously been studied by only a few authors (*De Roetth* 1957 *Limburg et al* 1952) Without specifying the criteria of the grading *De Roetth* (1957) divided her material of human epidermoid carcinomas of cervix into tumours of grade I–III Otherwise no attempts seem to have been made to correlate the metabolism of these tumours with the clinical stage or the histological differentiation of the material The present investigation was carried out in order to elucidate whether such a correlation exists between the glycolytic and respiratory metabolism of cervical carcinomas and the clinical and the histological findings.

Material and Method

Tissues from a total of 44 patients were examined including 18 cases of malignant cervical epithelium (microscopic diagnosis carcinoma planocellulare) and 19 cases of non-malignant cervical epithelium. There were a further 3 patients in whom a review of the histological preparations showed no invasive growth but cellular atypia and 4 patients with a clinical histo-pathological diagnosis of cervical cancer but in whom the biopsy specimens showed no tumour tissue on histological examination. The average age of the patients with cervical carcinoma was 53 9 years, while that of the patients with non-malignant disorders was 42.9 years.

All patients were admitted to the department of gynaecology at the Bispebjerg Hospital for surgical treatment. The malignant cases were treated by extended Wertheim operation while the non-malignant cases were treated by total hysterectomy A microscopic diagnosis was available for all patients prior to operation

Immediately after resection of the tissues suitable specimens were taken from the portio and placed in an ice cooled nutrient substrate consisting of a modified Eagle s medium (*Eagle* 1959) to which 20 % foetal calf serum was added. The tissue was then transported to the laboratory as rapidly as possible The time

elapsing from the moment of removal of the tissue till the first manometric reading rarely exceeded one hour Several authors (*De Roetth* 1957 *Macbeth*, 1962 *Roskelley et al.* 1943) have discussed the significance of the time factor for respiration and glycolysis, and found that storage for as much as 6 hours at 7 centigrade resulted in practically no noticeable fall in respiration and glycolysis.

Tissue respiration. After arrival at the laboratory the tissue was cut into thin slices with a maximum thickness of 0.5 mm, taking care to include as little connective tissue as possible. During the cutting, representative pieces of tissue were removed for histology These were fixed in 10 % formalin and stained with haematoxylin-eosin.

Tissue respiration was measured by conventional Warburg technique as described by *Umbreit* (1957) The sliced tissue was placed in Warburg flasks containing 0.2 ml 20 % KOH in the centre wells. Three Warburg flasks were used in each experiment, double determinations being made under aerobic conditions and single determinations under anaerobic conditions. Each flask contained an amount of tissue corresponding to 30–40 mg dry weight. The reaction medium was 2.3 ml of a Krebs-Ringer phosphate solution containing NaCl (0 154 M) KCl (0 154 M) $CaCl_2$ (0 11 M) $MgSO_4$, 7 H_2O (0 154 M) $NaHCO_3$ (0 154 M) and 0 1 M phosphate buffer pH 7 4 Immediately before use Tris (tris-(hydroxymethyl)-aminomethane) and glucose in amounts yielding final concentrations of 30 mM and 10 mM respectively were added t the reaction medium, and the flasks were left on ice for 15 minutes for equilibration

After equilibration, samples were withdrawn for glucose and lactate determination, and the total volume was adjusted to 2 ml per flask The flasks were then attached to the manometers, and after gassing with oxygen or nitrogen for 2 minutes, the stopcocks were closed and the flasks placed in the water bath at 37 About 20 minutes later – after temperature equilibration—the first reading was made Subsequent readings were made at 10–15 minutes intervals for a total period of 2 hours. At the end of the experiment samples were once more withdrawn for glucose and lactate determination

cellularity index which is a measure of the percentage of tumour cells counted in histological slides.

The metabolism of cervical carcinomas in women has previously been studied by only a few authors (*De Roetth* 1957 *Limburg et al* 1952) Without specifying the criteria of the grading *De Roetth* (1957) divided her material of human epidermoid carcinomas of cervix into tumours of grade I-III. Otherwise no attempts seem to have been made to correlate the metabolism of these tumours with the clinical stage or the histological differentiation of the material The present investigation was carried out in order to elucidate whether such a correlation exists between the glycolytic and respiratory metabolism of cervical carcinomas and the clinical and the histological findings.

Material and Method

Tissues from a total of 44 patients were examined, including 18 cases of malignant cervical epithelium (microscopic diagnosis. carcinoma planocellulare) and 19 cases of non-malignant cervical epithelium There were a further 3 patients in whom a review of the histological preparations showed no invasive growth but cellular atypia and 4 patients with a clinical histo-pathological diagnosis of cervical cancer but in whom the biopsy specimens showed no tumour tissue on histological examination. The average age of the patients with cervical carcinoma was 53.9 years, while that of the patients with non malignant disorders was 42.9 years

All patients were admitted to the department of gynaecology at the Bispebjerg Hospital for surgical treatment The malignant cases were treated by extended Wertheim operation while the non malignant cases were treated by total hysterectomy A microscopic diagnosis was available for all patients prior to operation

Immediately after resection of the tissues suitable specimens were taken from the portio and placed in an ice cooled nutrient substrate consisting of a modified Eagle s medium (*Eagle* 1959) to which 20 % foetal calf serum was added. The tissue was then transported to the laboratory as rapidly as possible. The time

elapsing from the moment of removal of the tissue till the first manometric reading rarely exceeded one hour. Several authors (*De Roetth* 1957, *Macbeth* 1962, *Roskelley et al.*, 1943) have discussed the significance of the time factor for respiration and glycolysis, and found that storage for as much as 6 hours at 7° centigrade resulted in practically no noticeable fall in respiration and glycolysis.

Tissue respiration. After arrival at the laboratory, the tissue was cut into thin slices with a maximum thickness of 0.5 mm, taking care to include as little connective tissue as possible. During the cutting, representative pieces of tissue were removed for histology. These were fixed in 10 % formalin and stained with haematoxylin-eosin.

Tissue respiration was measured by conventional Warburg technique as described by *Umbreit* (1957). The sliced tissue was placed in Warburg flasks, containing 0.2 ml 20 % KOH in the centre wells. Three Warburg flasks were used in each experiment, double determinations being made under aerobic conditions and single determinations under anaerobic conditions. Each flask contained an amount of tissue corresponding to 30–40 mg dry weight. The reaction medium was 2.3 ml of a Krebs-Ringer phosphate solution, containing NaCl (0.154 M), KCl (0.154 M), $CaCl_2$ (0.11 M), $MgSO_4 \cdot 7 H_2O$ (0.154 M), $NaHCO_3$ (0.154 M) and 0.1 M phosphate buffer, pH 7.4. Immediately before use, Tris (tris-(hydroxymethyl)-aminomethane) and glucose in amounts yielding final concentrations of 30 mM and 10 mM, respectively, were added to the reaction medium, and the flasks were left on ice for 15 minutes for equilibration.

After equilibration, samples were withdrawn for glucose and lactate determination, and the total volume was adjusted to 2 ml per flask. The flasks were then attached to the manometers, and after gassing with oxygen or nitrogen for 2 minutes, the stopcocks were closed and the flasks placed in the water bath at 37°. About 20 minutes later—after temperature equilibration—the first reading was made. Subsequent readings were made at 10–15 minutes intervals for a total period of 2 hours. At the end of the experiment, samples were once more withdrawn for glucose and lactate determination.

Glucose determination The glucose content was determined enzymatically by the specific oxidation of glucose to gluconolactone catalyzed by glucose oxidase. Gluconolactone reacts with H_2O to form H_2O_2, which in the presence of peroxidase will change o-dianisidine into a red-brown stain which can be measured spectrophotometrically at 436 mμ (*Hugget* 1957)

Lactic acid determination The lactic acid content was determined enzymatically (*Horn* 1956) by spectrophotometric measurements at 340 mμ of the NADH production in 1 cm cuvettes, containing 2 ml glycine (0 5 M)/hydrazine (0 4 M) buffer (pH 9 0) 0 1 ml of the deproteinized sample 0 03 ml lactic acid dehydrogenase (LDH 2 mg enzyme protein per ml) and 0.2 ml NAD (0 027 M)

The reagents used for glucose and lactate determinations were all purchased from Boehringer & Söhne Mannheim Germany

Dry weight determination At the end of the experiments the dry weight of the tissue slices was determined by heating at 90 centigrade until constant weight was reached.

Results

The results obtained were calculated as shown in Table I, in which the various quotients commonly used in studies of this type are presented

Table II shows the values obtained with malignant tissue This table also indicates the clinical stage and differentiation, as well as possible lymph-node metastases or recurrence in the pelvis. With respect to the degree of differentiation, a distinction was drawn between highly moderately and poorly or undifferentiated cervical carcinoma. The highly differentiated tissue was characterized by large abnormal epithelial cells, epithelial pearls, keratinisation and low mitotic index The moderately differentiated tissue showed many pleomorphic cells no epithelial pearls but occasional keratinisation, and a moderately high mitotic index The poorly or undifferentiated tissue was characterized by small basophilic cells with a high nucleus-cell ratio and a high mitotic index (*Wentz et al* 1965)

Table III shows the results obtained with non-malignant tissues

Table I

Q_{O_2}	oxygen uptake calculated as 10^{-8} moles per mg dry weight per hour
$Q_G^{O_2}$	glucose uptake calculated as 10^{-8} moles per mg dry weight per hour under aerobic conditions.
$Q_L^{O_2}$	lactic acid production calculated as 10^{-8} moles per mg dry weight per hour under aerobic conditions.
$\frac{Q_L^{O_2}}{Q_{O_2}}$	Warburg coefficient.
$Q_G^{N_2}$	glucose uptake calculated as 10^{-8} moles per mg dry weight per hour under anaerobic conditions.
$Q_L^{N_2}$	lactic acid production calculated as 10^{-8} moles per mg dry weight per hour under anaerobic conditions.
$\frac{Q^{N_2}}{Q^{N_2}}$	ratio between lactic acid production and glucose uptake under anaerobic conditions.
$\frac{Q^{O_2}}{Q^{O_2}}$	ratio between lactic acid production and glucose uptake under aerobic conditions.
M O C	Meyerhof oxidation coefficient $\frac{Q^{N_2} \quad Q^{O_2}}{1\ 3 \quad Q_{O_2}}$
P P E	Percentage Pasteur effect $\frac{100\,(Q \quad - Q_L^{O_2})}{Q^{N_2}}$

It appears from Tables II and III that both respiration and glycolysis had a higher level in malignant tissue than in non-malignant tissue The mean values and the 95 per cent confidence limits are shown in Tables IV and V from which it is seen that the oxygen uptake the aerobic and anaerobic glucose consumption and the lactic acid accumulation were all significantly higher in malignant tissue On the other hand, no difference was found between the Warburg quotient $\frac{Q^{O_2}}{Q_{O_2}}$ in malignant and non-malignant tissue, the value being 1 98 in both cases. The ratio between lactate accumulation and glucose consumption was somewhat greater in non-malignant tissue than in malignant tissue However it was only under anaerobic conditions that this difference was found to be statistically significant.

Glucose determination The glucose content was determined enzymatically by the specific oxidation of glucose to gluconolactone catalyzed by glucose oxidase. Gluconolactone reacts with H_2O to form H_2O_2, which in the presence of peroxidase will change o-dianisidine into a red brown stain which can be measured spectrophotometrically at 436 mμ (*Hugget* 1957)

Lactic acid determination The lactic acid content was determined enzymatically (*Horn* 1956) by spectrophotometric measurements at 340 mμ of the NADH-production in 1 cm cuvettes, containing 2 ml glycine (0.5 M)/hydrazine (0 4 M) buffer (pH 9 0) 0 1 ml of the deproteinized sample 0 03 ml lactic acid dehydrogenase (LDH 2 mg enzyme protein per ml) and 0.2 ml NAD (0 027 M)

The reagents used for glucose and lactate determinations were all purchased from Boehringer & Söhne Mannheim Germany

Dry weight determination At the end of the experiments the dry weight of the tissue slices was determined by heating at 90 centigrade until constant weight was reached.

Results

The results obtained were calculated as shown in Table I, in which the various quotients commonly used in studies of this type are presented.

Table II shows the values obtained with malignant tissue This table also indicates the clinical stage and differentiation as well as possible lymph-node metastases or recurrence in the pelvis. With respect to the degree of differentiation, a distinction was drawn between highly moderately and poorly or undifferentiated cervical carcinoma The highly differentiated tissue was characterized by large abnormal epithelial cells, epithelial pearls, keratinisation and low mitotic index The moderately differentiated tissue showed many pleomorphic cells no epithelial pearls but occasional keratinisation, and a moderately high mitotic index The poorly or undifferentiated tissue was characterized by small basophilic cells with a high nucleus-cell ratio and a high mitotic index (*Wentz et al* 1965)

Table III shows the results obtained with non-malignant tissues

Table III. Non-Malignant Cervical Epithelium (for Symbols see Table I)

Experiment No	Q_{O_2}	$Q_G^{O_2}$	Q^{O_2}	$\frac{Q_L^{O_2}}{Q_{O_2}}$	$Q_G^{N_2}$	Q^{N_2}	$\frac{Q_L^{N_2}}{Q_G^{N_2}}$	$\frac{Q_L^{O_2}}{Q_G^{O_2}}$	M.O.C.	P.P.E.
6	5.98	2.01	5.27	0.89				2.63		
7	8.77	3.52	5.82	0.67				1.66		
8	3.44	2.00	3.74	1.22				1.87		
9	6.28	2.52	7.97	1.34				5.85		
10	4.00	2.80	7.37	2.05	8.26	15.71	1.90	2.64	6.27	53.10
11	2.39	2.79	6.71	3.01		14.22		2.52	9.39	52.81
12	2.93	2.47	4.98	2.64	5.91	11.47	1.94	2.02	6.62	56.58
15	6.39	5.33	11.41	2.01	5.02	16.22	3.23	2.86	2.26	29.65
22	6.05	5.91	9.24	1.52	6.33	13.44	2.12	1.58	2.08	31.25
26	6.11	4.77	8.74	1.49	7.99	16.15	2.02	1.84	3.63	45.88
33	2.68	1.96	7.29	2.72	4.56	13.57	2.98	3.72	7.08	46.28
35	4.41	3.73	8.54	1.96	6.55	16.92	2.58	2.31	5.70	49.53
36	2.62	1.67	6.03	2.37	4.29	11.16	2.60	3.90	5.90	45.50
37	3.44	2.77	8.23	2.65	4.63	13.78	2.98	2.99	4.83	40.28
41	2.97	1.57	6.40	2.17	2.05	9.16	4.47	4.12	2.79	30.13
42	5.14	4.35	8.87	1.75	7.92	22.84	2.88	2.36	8.17	61.18
44	4.96	4.17	9.84	1.99	6.08	22.12	3.64	2.36	7.44	55.52
45	2.98		9.27	3.30		19.51			10.34	52.49
46	6.11	3.82	10.90	1.78	8.48	23.02	2.71	2.84	5.94	52.65

Table IV. *Mean Values under Aerobic Conditions*

	Malignant Tissue	Non-Malignant Tissue
Q_{O_2}	10.18 ± 1.52 (20)	4.61 ± 0.71 (19)
Q^{O_2}	10.03 ± 1.99 (18)	3.23 ± 0.53 (18)
Q^{O_2}	18.09 ± 3.16 (18)	7.70 ± 0.81 (19)
$\frac{Q^{O_2}}{Q_{O_2}}$	1.98 ± 0.36 (18)	1.98 ± 0.28 (19)
$\frac{Q^{O_2}}{Q^{O_2}}$	2.06 ± 0.37 (18)	2.88 ± 0.46 (18)

For symbols see Table I.
Numbers of patients in brackets.

Table II. *(for Symbols see Table I)*

Malignant Cervical Epithelium

Experiment No	Clinical Stage	Degree of Differentiation	Metastases or Local Recurrence	Q_{O_2}	$Q_G^{O_2}$	$Q_L^{O_2}$	$\frac{Q_L^{O_2}}{Q_{O_2}}$	$Q_G^{N_2}$	Q_L^N	$\frac{Q_L^{N_2}}{Q_G^{N_2}}$	$\frac{Q_L^{O_2}}{Q_G^{O_2}}$	M.O C	P P.E.
3	recurrent c colli ut.	highly	+ recurr − gll	9 82	2 93	9.03	0.96				3 26		
4	I b	moderately	− gll	3 24	1.39	5 97	1.39				5.32		
14	II III	moderately	− gll	10 61	12.41	21 18	1.95	15 11	27.88	1.85	1 71	1.89	24 03
16	recurrent c. colli ut	poorly	+ recurr + gll	9 72	9 74	17 30	1 79	18.45	35.58	1 93	1 79	5.64	51.38
18	II III	poorly	− gll	10 03	9.06	16 60	1 66	12.63	26 45	2 09	1 83	2.95	37.24
19	I b	poorly	gll	12.78	9.83	16.24	1.30	12.67	28.34	2.04	1 66	2.84	42.70
20	I b	modera ely	− gll	12 72	8.43	18.38	1 45	15.38	35.05	2.28	2.18	3 93	47.56
23	II b	poorly	gll	10.65	8 89	16 55	1.54	19.50	26.33	1.35	1 68	2.75	37 14
24	II a	poorly	+ gll	6.35	8 26	16 25	2.58	10 44	20 06	1.92	1.97	1 80	18.99
27	I b	moderately	− gll	17 61	11.30	19.05	1.08				1 69		
28	I b	poorly	− gll	9 79	13 79	25.06	2.56	20 19	39 69	1.97	1.82	4 49	36.90
29	I b	poorly	− gll	15 17	9.67	16 32	1 10	19 63	40 72	2.07	1.69	4.82	59.90
32	II a	moderately	− gll	5 40	11 26	16 12	3 09	8.75	16.53	1 89	1.51	0.23	2.48
38	I b	highly	+ recurr − gll	6 37	9.36	16.28	2 68	13 63	23.34	1 71	1 75	3.33	30.25
39	II a	poorly	+ gll	13 97	14.58	23 12	1 61	20.96	39.51	1.89	1.59	3.52	41 48
40	II b	poorly	gll	8.37	7 11	13 76	1 65	12.12	24.00	1 98	1.94	3 67	42.67
43	recurrent c. colli ut.	poorly	+ ecurr − gll	5 22	7.86	14 93	2.84	13.85	29.20	2.11	1.90	8.20	48.87
47	II a	poorly	− gll	9.95	24 60	43 48	4.38	28 40	53 81	1 89	1 77	3 11	20.00

Table III *Non-Malignant Cervical Epithelium (for Symbols see Table I)*

Experiment No.	Q_{O_2}	$Q_G^{O_2}$	$Q_L^{O_2}$	$\frac{Q_L^{O_2}}{Q_{O_2}}$	$Q_G^{N_2}$	$Q_L^{N_2}$	$\frac{Q_L^{N_2}}{Q_G^{N_2}}$	$\frac{Q^{O_2}}{Q_G^{O_2}}$	M.O.C.	P.P.E.
6	5.98	2.01	5.27	0.89				2.63		
7	8.77	3.52	5.82	0.67				1.66		
8	3.44	2.00	3.74	1.22				1.87		
9	6.28	2.52	7.97	1.34				5.85		
10	4.00	2.80	7.37	2.05	8.26	15.71	1.90	2.64	6.27	53.10
11	2.39	2.79	6.71	3.01		14.22		2.52	9.39	52.81
12	2.93	2.47	4.98	2.64	5.91	11.47	1.94	2.02	6.62	56.58
15	6.39	5.33	11.41	2.01	5.02	16.22	3.23	2.86	2.26	29.65
22	6.05	5.91	9.24	1.52	6.33	13.44	2.12	1.58	2.08	31.25
26	6.11	4.77	8.74	1.49	7.99	16.15	2.02	1.84	3.63	45.08
33	2.68	1.96	7.29	2.72	4.56	13.57	2.98	3.72	7.06	46.28
35	4.41	3.73	8.54	1.96	6.55	16.92	2.58	2.31	5.70	49.53
36	2.62	1.67	6.03	2.37	4.29	11.16	2.60	3.90	5.90	45.50
37	3.44	2.77	8.23	2.65	4.63	13.78	2.98	2.99	4.83	40.28
41	2.97	1.57	6.40	2.17	2.05	9.16	4.47	4.12	2.79	30.13
42	5.14	4.35	8.87	1.75	7.92	22.84	2.88	2.36	8.17	61.16
44	4.96	4.17	9.84	1.99	6.08	22.12	3.64	2.36	7.44	55.52
45	2.98		9.27	3.30		19.51			10.34	52.49
46	6.11	3.82	10.90	1.78	8.48	23.02	2.71	2.84	5.94	52.65

Table IV *Mean Values under Aerobic Conditions*

	Malignant Tissue	Non-Malignant Tissue
Q_{O_2}	10.18 ± 1.52 (20)	4.61 ± 0.71 (19)
Q^{O_2}	10.03 ± 1.99 (18)	3.23 ± 0.53 (18)
Q^{O_2}	18.09 ± 3.16 (18)	7.70 ± 0.81 (19)
$\frac{Q^{O_2}}{Q_{O_2}}$	1.98 ± 0.36 (18)	1.98 ± 0.28 (19)
$\frac{Q_L^{O_2}}{Q^{O_2}}$	2.06 ± 0.37 (18)	2.88 ± 0.46 (18)

For symbols see Table I
Numbers of patients in brackets

Table V *Mean Values under Anaerobic Conditions*

	Malignant Tissue	Non Malignant Tissue
$Q_G^{N_2}$	16.91 ± 2.42 (15)	6.01 ± 0 93 (13)
Q_L^N	31 10 ± 4.38 (15)	15.95 ± 1.95 (15)
$\frac{Q_L^{N_2}}{Q_G^N}$	1.95 ± 0 10 (15)	2.77 ± 0.36 (13)

For symbols see Table I.
Numbers of patients in brackets.

Tables II and III also give the percentage Pasteur effect and the Meyerhof oxidation quotient Both a higher percentage Pasteur effect and a higher Meyerhof coefficient were found in non-malignant tissue compared with malignant tissue

Finally Tables VI and VII show the values for 3 tissue specimens showing cellular atypia and the values for the 4 cases with the clinical-histo-pathological diagnosis of cervical cancer but where the biopsy specimens showed no tumour tissue. The values in these tables did not differ from the values for normal cervical epithelium.

In Table VIII clinical stage I has been compared with clinical stages II–III and in Table IX patients with metastases or recurrence have been compared with patients without metastases or recurrence The tables show that there was no significant correlation between the clinical stage and the metabolic findings Nevertheless a tendency to rising Warburg quotient was found in stages II and III

Discussion and Conclusion

The results show that in the present study of the cervical epithelium both a higher aerobic glycolysis and a higher anaerobic glycolysis were found in malignant tissue than in non-malignant tissue This is in general agreement with previous studies on human tissue as reviewed by *Aisenberg* (1961) Furthermore, a higher oxygen uptake was found in malignant cervical epithelium.

Table VI Tissue with Cellular Atypia

Experiment No	Q_{O_2}	$Q_G^{O_2}$	Q^{O_2}	$\frac{Q^{O_2}}{Q_{O_2}}$	$\frac{Q_L^{O_2}}{Q_G^{O_2}}$	Q^{N_2}	$Q_L^{N_2}$	$\frac{Q_L^{N_2}}{Q_G^{N_2}}$	M.O.C.	P.P.E.
17	6.50	5.25	11.85	1.82	2.26	7.85	22.30	2.84	4.32	36.77
21	3.13	3.34	7.72	2.54	2.38	3.57	12.21	2.19	4.02	36.70
34	4.16	4.60	8.64	2.36	2.09	5.92	15.23	2.57	4.82	46.86

Symbols see Table I

Table VII Tissue from Patient with Cervical Carcinoma but Microscopy Showed No Tumour Tissue

Experiment No	Q_{O_2}	Q^{O_2}	Q^{O_2}	$\frac{Q^{O_2}}{Q_{O_2}}$	$\frac{Q_L^{O_2}}{Q_G^{O_2}}$	Q^{N_2}	$Q_L^{N_2}$	$\frac{Q^{N_2}}{Q_G^{N_2}}$	M.O.C.	P.P.E.
5	8.42	4.23	7.17	0.87	1.71					
13	3.12	2.26	5.89	1.90	2.76	2.53	9.15	3.61	3.13	35.63
25	4.78	[illegible]	12.93	2.70	1.66	10.48	17.63	1.08	2.96	26.66
31		2.41	4.17		1.82	4.59	6.74	1.47		38.10

Symbols see Table I

Table VIII Comparison Between Clinical Stage I and Stages II–III or Recurrence

	Q_{O_2}	Q^{O_2}	$Q_L^{O_2}$	$\frac{Q_L^{O_2}}{Q_{O_2}}$	$Q_G^{N_2}$	Q^{N_2}	$\frac{Q_L^{N_2}}{Q_G^{N_2}}$	$\frac{Q^{O_2}}{Q_G^{O_2}}$
Stage I	11.10 ± 3.67	9.11 ± 2.79	16.76 ± 4.17	1.65 ± 0.50	16.30 ± 3.28	33.43 ± 7.19	2.05 ± 0.49	2.30 ± 0.99
Stage II–III or recurrence	9.10 ± 2.12	10.61 ± 3.02	18.94 ± 4.87	2.19 ± 0.85	16.03 ± 3.42	29.94 ± 6.21	1.89 ± 0.39	1.90 ± 0.27

Symbols see Table I

Table V *Mean Values under Anaerobic Conditions*

	Malignant Tissue	Non-Malignant Tissue
Q_G^{N}	16.91 ± 2.42 (15)	6 01 ± 0.93 (13)
$Q_L^{N_2}$	31 10 ± 4.38 (15)	15.95 ± 1.95 (15)
$\frac{Q_L^{N}}{Q_G^{N_2}}$	1.95 ± 0 10 (15)	2.77 ± 0.36 (13)

For symbols see Table I.
Numbers of patients in brackets.

Tables II and III also give the percentage Pasteur effect and the Meyerhof oxidation quotient Both a higher percentage Pasteur effect and a higher Meyerhof coefficient were found in non-malignant tissue compared with malignant tissue

Finally Tables VI and VII show the values for 3 tissue specimens showing cellular atypia and the values for the 4 cases with the clinical histo-pathological diagnosis of cervical cancer but where the biopsy specimens showed no tumour tissue The values in these tables did not differ from the values for normal cervical epithelium.

In Table VIII clinical stage I has been compared with clinical stages II–III and in Table IX patients with metastases or recurrence have been compared with patients without metastases or recurrence The tables show that there was no significant correlation between the clinical stage and the metabolic findings Nevertheless a tendency to rising Warburg quotient was found in stages II and III

Discussion and Conclusion

The results show that in the present study of the cervical epithelium both a higher aerobic glycolysis and a higher anaerobic glycolysis were found in malignant tissue than in non malignant tissue This is in general agreement with previous studies on human tissue as reviewed by *Aisenberg* (1961) Furthermore a higher oxygen uptake was found in malignant cervical epithelium

in almost all malignant samples, while all non-malignant samples showed values clearly below the malignant average. Thus the metabolic and the histological observations agreed well, indicating that metabolic studies do not have much to offer from a practical diagnostic point of view which cannot be obtained by conventional histological procedures.

Also from a prognostic point of view such studies seem to be of limited value. Apart from a tendency to higher Warburg quoti ents in cervical carcinoma of stages II–III as compared with stage the degree of malignancy as expressed by clinical stage and differentiation showed no correlation with the values found.

The patients were reviewed for occurrence of metastases and signs of relapse but no greater incidence of metastases was found in patients in whom the metabolic and respiratory studies showed high values.

No differences were found between the values observed in tissues with cellular atypia, non-malignant tissue portions of cervical carcinoma, and non-malignant tissue. This does not agree with the results obtained by *Briand* (1967) who found malignant Warburg quotients between 2.0 and 3.1 in human portio biopsies which on microscopy showed various degrees of atypia.

In their studies of the LDH-isoenzyme pattern in preinvasive carcinoma of the cervix, *Larner Turner* and *Way* (1966) found isoenzyme changes suggestive of malignancy even in regions where microscopic study provided no support for abnormalities. Studies by *Langvad* of the isoenzyme distribution of LDH in tumour-bearing colon and in bronchogenic carcinoma (1968) likewise showed that changes characteristic of malignant tissue can be found in regions with no morphological abnormalities.

The studies of *Larner et al.* (1966) *Briand* (1967) and *Langvad* (1968) suggest that even at an early stage in the development of cancer when it is still not possible to demonstrate morphological changes in the direction of malignancy metabolic and enzymatic changes occur which are characteristic of tumour tissue growing invasively

No support for this hypothesis could be found in the present study This, however does not exclude that early enzymatic changes might be revealed by more sensitive and specific methods

Table IX. *Comparison Between Patients with Metastases (or Recurrence) and Patie*
Without Metastases

	Q_{O_2}	$Q_G^{O_2}$	$Q_L^{O_2}$	$\frac{Q_L^{O_2}}{Q_{O_2}}$	$Q_G^{N_2}$	$Q_L^{N_2}$	$\frac{Q_L^{N_2}}{Q_G^{N_2}}$	$\frac{Q_L^{O_2}}{Q_G^{O_2}}$
+ metastases	8.56 ± 2.68	8.79 ± 3.08	16.15 ± 3.77	2.08 ± 0.60	15.47 ± 3.98	29.54 ± 7.74	1.91 ± 0.22	2.0 ± 0
÷ metastases	10.53 ± 2.01	10.65 ± 2.79	19.06 ± 4.62	1.93 ± 0.52	16.44 ± 3.29	31.88 ± 6.16	1.96 ± 0.38	2.0 ± 0

Symbols see Table I.

These observations are in agreement with the results obtained by *De Roetth* (1957) *Macbeth* (1962) and *Dickens Weil* (1943) but not with the results reported by *Dreyfuss* (1940)

Briand's studies of human biopsies (1967) indicate that the so-called Warburg quotient *i.e.* the rate of lactate accumulation to oxygen consumption $\frac{Q_L^{O_2}}{Q_{O_2}}$ is the most reliable metabolic criterion of malignancy Thus, 78 per cent of malignant biopsies showed Warburg quotients above 1.5 while similar values were found in only 8 per cent of non malignant biopsies. In the present investigation 75 and 66 per cent of the non malignant and the malignant biopsies respectively showed Warburg quotients above 1.50 The mean value in the two groups was the same, *i.e* [?]98 Thus the Warburg quotient cannot be used as a criterion of malignancy in cervical carcinoma.

On the other hand both respiration, aerobic, and anaerobic glycolysis were strongly elevated in the malignant material. This may be due to the fact that *there is a greater cell density in the* malignant tissue No attempts were made in the present study to determine the carcinoma-cellularity-index, since no accurate corrections of the results can be obtained in this way However the fact that the metabolic balance was the same in the different groups of tissue suggests that the increased respiration and glycolysis are due exclusively to differences in cellularity

The increased rates of respiration and glycolysis were found

Lactate Dehydrogenase Isoenzyme Patterns in the Tumour-Bearing Colon. International Journal of Cancer 3 17 1968

Lerner A. L., Turner D. M. and *Way S. A.* Lancet II 814 1966

Limburg, H. and *Uhlmann, G.*, Ztschr f Krebsforschung, 58, 478, 1952

Macbeth R. A. L. and *Bekesi J. G.*, Cancer Res. 22, 244 1962

Roskelley R. C. Mayer N. Horwitt B. N and *Salter W. F.* J Clin. Invest. 22 743 1943

Umbreit W. W. Burris R. H and *Stauffer J. F.* Manometric Techniques in Tissue Metabolism, 3d ed. Minneapolis, Burgess Publishing Co 1957

Warburg, O. Metabolism of Tumours (Translated by F. Dickens) London, Arnold Constable, 1930

Wenner W. B. and *Lewis G. C.*, Obst. & Gynec. 26 228, 1965

Received on Dec 20 1967

SUMMARY

Oxygen consumption and aerobic and anaerobic glycolysis were studied in uterine cervix biopsies from 44 patients with non-malignant and malignant disease. The oxygen uptake was measured by the conventional Warburg technique and glucose and lactate content by specific enzymatic methods

The metabolic balance as expressed by the Warburg quotient $\frac{Q_L^{O_2}}{Q_{O_2}}$ was found to be elevated both in non-malignant and malignant biopsies but the absolute values of oxygen consumption, glucose consumption and lactate production were much higher in malignant tissues No correlation was established between these metabolic parameters and the clinical stage of the disease.

This investigation was supported by a personal grant from The Danish Cancer Society and by the Daell Foundation

Acknowledgements

The author wishes to thank Physician-in-Chief E. Bredahl The Department of Pathology Bispebjerg Hospital and Physician-in Chief G Hjorth The Department of Pathology Finseninstitutet, for kind advice in evaluating the microscopic slides.

REFERENCES

Aisenberg, A. C The Glycolysis and Respiration of Tumours, New York Academic Press 1961

Briand P Thesis 1967 (to be published)

De Roetth H Cancer Res *17* 833 1957

Dickens F and *Patey D H* Lancet *219* 1229 1930

Dickens F and *Weil Malherbe H* Cancer Res. *3* 73 1943

Dreyfus M L. Amer J of Cancer *38* 551 1940

Eagle H Science *130* 432 1959

Horn H D and *Bruns F H* Biochem. Biophys Acta *21* 378 1956

Hugget A. St G and *Nixon D A.* Biochem J *66* 12 1957

Journal of The International Federation of Gynecology & Obstetrics, *3* 204 1965

Langvad E. Lactate Dehydrogenase Isoenzyme Patterns in Bronchogenic Carcinoma. European Journal of Cancer *4* 107 1968

Lactate Dehydrogenase Isoenzyme Patterns in the Tumour-Bearing Colon. International Journal of Cancer 3 17 1968
Letner A. L., Turner D. M. and *Way S. A.* Lancet II 814 1966
Lineberg, H. and *Uhlmann G* Ztschr f Krebsforschung, 58, 478, 1952
Macbeth, R. A. L. and *Bekesi J G* Cancer Res. 22, 244 1962
Roskelley R. C Mayer N Horwitt B N and *Salter W F.*, J Clin. Invest. 22 743 1943
Umbreit W W Burris R. H and *Stauffer J E.* Manometric Techniques in Tissue Metabolism, 3d ed Minneapolis, Burgess Publishing Co 1957
Warburg, O Metabolism of Tumours (Translated by F Dickens) London, Arnold Constable, 1930
Wentz W B. and *Lewis G. C.*, Obst. & Gynec. 26 228, 1965

Received on Dec. 20 1967

Metsälä Perttu and Nieminen Usko, Acta obst. et gynec. scandinav. 47 482, 1968

From the I and II Clinics of Obstetrics and Gynaecology (Professor Aarno Turunen and Professor Paavo Vara) Helsinki University Central Hospital Finland

TRAUMATIC LESIONS OF THE VAGINA

BY

PERTTU METSÄLÄ AND USKO NIEMINEN

Nonobstetrical traumatic lesions of the vagina commonly occur in connection with coitus. Several investigators have attempted to elucidate the mechanisms which give rise to such lesions. *Ralm* reviewed the literature published up to 1925 and gave an interpretation based on his own case histories, of the aetiology of coital lesions of the vagina. He claimed that the most important factor conducive to rupture of the vaginal vault was abnormal muscle spasm in the vaginal wall which led to a reduction in vaginal volume and a lowering of its elasticity According to him, the contraction is linked to the intense sexual feeling of the woman, but needs as a prerequisite an additional factor probably of mental origin

He further mentions a gross inequality in the proportions of the penis and vagina the postpuerperal state hypoplastic sexual organs inflammatory changes in the vicinity of the vagina, retroversioflexion of the uterus and exceptional coital positions. Ruptures below the vaginal vault occur according to *Ralm* usually in connection with defloration, often during forcible coitus The most important reason for this trauma is weakness of of the vaginal wall caused by genital hypoplasia or alterations due to old age

In 1959 *Klaue* presented 56 cases with traumatic lesions of the vaginal vault According to him, these are caused by and are the consequence of excessive sexual stimulation of the woman. *Engel* (1965) has recently presented a list of the causes of coital lesions

1. Excessively active involvement of the woman
2. Mental factors.
   a. intense sexual stimulation and consequent elevation of pain threshold
   b. fear of interruption by an outsider
   c. coitus following a long pause in sexual activity
3. Intoxication
4. Clumsiness
5. Exceptional coital positions
6. Anatomical disproportion
7. Violence
8. Pregnancy postpartal or postmenstrual state
9. Increased vulnerability of the organ (e.g. recent vaginal operation)
10. Vaginal cramps.

Purnell (1965) has presented a review of the literature and three examples of his own of apparently spontaneous rupture of the vaginal vault in the absence of intravaginal trauma. All cases recorded have been postmenopausal, with atrophy of the vaginal walls. Vaginal rupture occurred at a moment when the abdominal muscles were suddenly brought into action as during lifting, coughing, falling or defaecation. The abrupt increase in intra abdominal pressure thus produced, acting through the pouch of Douglas on a weakened posterior fornix, is thought by the writer to be the most important factor in bringing about a rupture.

It seems obvious that an elucidation of the mechanisms of coital lesions has to be based on the physical events actually taking place in the sexually excited female organism. An outstanding source of information in this respect has recently become available in the published results of direct observations and physical measurements carried out between 1939–1965 by *Masters* and *Johnson* in women stimulated by various sorts of coital and noncoital manipulations.

The human female cycle of sexual response is divided into four separate phases

1. the excitement phase
2. the plateau phase
3. the orgasmic phase and
4. the resolution phase

Anatomically the unstimulated vagina is potential rather than an actual space with the anterior and posterior walls essentially contiguous. During the

Metsälä, Perttu and Nieminen, Usko. Acta obst. et gynec. scandinav. 47: 482, 1968

From the I and II Clinics of Obstetrics and Gynaecology (Professor Aarno Turunen and Professor Paavo Vara) Helsinki University Central Hospital Finland

TRAUMATIC LESIONS OF THE VAGINA

BY

PERTTU METSÄLÄ AND USKO NIEMINEN

Nonobstetrical traumatic lesions of the vagina commonly occur in connection with coitus. Several investigators have attempted to elucidate the mechanisms which give rise to such lesions. *Rahm* reviewed the literature published up to 1925 and gave an interpretation, based on his own case histories, of the aetiology of coital lesions of the vagina. He claimed that the most important factor conducive to rupture of the vaginal vault was abnormal muscle spasm in the vaginal wall, which led to a reduction in vaginal volume and a lowering of its elasticity. According to him, *the contraction is linked to the intense sexual feeling of the* woman but needs as a prerequisite an additional factor probably of mental origin.

He further mentions a gross inequality in the proportions of the penis and vagina, the postpuerperal state, hypoplastic sexual organs, inflammatory changes in the vicinity of the vagina, retroversioflexion of the uterus, and exceptional coital positions. Ruptures below the vaginal vault occur according to *Rahm* usually in connection with defloration, often during forcible coitus. The most important reason for this trauma is weakness of of the vaginal wall caused by genital hypoplasia or alterations due to old age.

In 1959 *Klauve* presented 56 cases with traumatic lesions of the vaginal vault. According to him, these are caused by and are the consequence of excessive sexual stimulation of the woman. *Engel* (1965) has recently presented a list of the causes of coital lesions.

Table I. *The Distribution in Age-Groups of 76 Vaginal Ruptures Necessitating Hospital Treatment During 1951 1965*

Age	18	18–20	21–30	31–40	41 50	51–60	61 70	71–80	81 90
Number	8	18	22	13	6	5	1	2	1
								Total	76

Table II. *Distribution of Vaginal Ruptures During Coitus According to Marital Status*

Marital Status	Unmarried	Married	Divorced	Widowed	Total
Number (per cent)	49 (73 %)	11 (16 %)	5 (8 %)	2 (3 %)	67

The youngest patients were two 15-year old girls. One suffered from profuse bleeding from a hymen ruptured during attempted coitus. In the other girl a 4 cm rupture was found in the vaginal vault she had been found in a confused and disoriented state in the street and could not produce any description of the causative events. The oldest patient was a 88-year old widow in whom the introduction of an indwelling speculum during colposcopic inspection produced a 4 cm rupture into the vaginal vault. The majority of the patients were of reproductive age 70 per cent of them were between 18 and 40 years old. The average age of menarche in the series was 14 0 years. The average age of menarche in Finland was reported 14.88 years in 1943 (*Vara*) and 13.31 years in 1966 (*Metsälä*) Most of the lesions (in 67 cases or in 88 per cent) had been acquired during coitus. Four of the nine noncoital lesions were produced by the diagnostic introduction of an indwelling speculum, one by a therapeutic syringe applicator introduced into the vagina, and a sixth by the introduction of a man s fist into the vagina In the latter case a 10 cm longitudinal rupture had been produced into the left-hand wall of the vagina, and several ruptures were apparent in the mucous membrane of the vulva. In the following three cases the vaginal rupture had been produced without forcible intervention inside the organ itself

Case 1 Age 44 years Married 22 years, 2 children The patient had slipped in front of moving streetcar which hit and fractured her left hip In the left-hand wall of the vagina rupture was found, beginning from the introitus.

Case 2 77 year old widow 2 children and 1 abortion Menopause at 44 years The patient had lost her balance and had fallen on the ground. This resulted

excitement phase there is lengthening and distension of the inner two-thirds of the vagina. Before termination of the excitement phase the vaginal canal is markedly expanded, and the cervix and corpus of uterus are pulled back and up into the false pelvis so that the cervix is removed from its normal resting position in direct contact with the posterior vaginal floor The sexually unstimulated vagina of nulliparous woman measures 2 cm in diameter in the transcervical plane. As sexual tensions mount transcervical vagina-wall expansion ranges from 5 75 to 6.25 cm. Vaginal length increases from to 8 cm in the unstimula ed vagina to 9.5–10.5 cm during excitement-phase response. Distended by an indwelling speculum the transcervical expansion reaches 6.75–7.25 cm and vaginal-length extension 11–1 cm with excitement-phase response.

During the plateau phase a minimal further increase in which and depth of the vagina occurs while a marked localized vasocongestive reaction develops in the outer third of the vagina. This specific area of the vagina, including the bulbus vestibuli and the labia minora, has been termed by *Masters* and *Johnson* the orgasmic platform.

During orgasm the orgasmic platform contracts strongly in a regularly recurring pattern. These contractions of the outer third of the vagina are the only physiologic responses of the vaginal canal that are confined to the orgasmic phase while the basic reaction of the inner two-thirds of the vagina is essentially expansive rather than constrictive in character

The localized vasocongestive reaction is rapidly dispersed with the onset of the resolution phase The inner two-thirds of the vagina slowly shrinks back to the collapsed, unstimulated state and the anterior wall and the cervix of the anteriorly positioned uterus descend toward the vaginal floor

In the light of these results and additional recently published data, we shall try to elucidate the mechanisms of causation of coital lesions on the basis of previously unpublished material studied by us

Material

We have studied the incidence and types of traumatic lesions of the vagina in the records from 19 1 to 1965 of the I and II Clinics of Obstetrics and Gynaecology Helsinki University Central Hospital. During these years 76 ruptures of the vagina have been treated in the hospital lesser lesions which have not necessitated hospital treatment, except at the policlinics, have not been included in the material There were althogether 329 637 policlinical consultations during these years and vaginal ruptures necessitating hospital treatment thus comprised 0.02 per cent of this total.

Table I *The Distribution in Age-Groups of 76 Vaginal Ruptures Necessitating Hospital Treatment During 1951 1965*

Age	18	18–20	21 30	31–40	41 50	51–60	61 70	71–80	81 90
Number	8	18	22	13	6	5	1	2	1
								Total	76

Table II *Distribution of Vaginal Ruptures During Coitus According to Marital Status*

Marital Status	Unmarried	Married	Divorced	Widowed	Total
Number (per cent)	49 (73 %)	11 (16 %)	5 (8 %)	2 (3 %)	67

The youngest patients were two 15-year old girls. One suffered from profuse bleeding from a hymen ruptured during attempted coitus. In the other girl a 4 cm rupture was found in the vaginal vault she had been found in a confused and disoriented state in the street and could not produce any description of the causative events. The oldest patient was a 88-year old widow in whom the introduction of an indwelling speculum during colposcopic inspection produced a 4 cm rupture into the vaginal vault. The majority of the patients were of reproductive age 70 per cent of them were between 18 and 40 years old. The average age of menarche in the series was 14 0 years. The average age of menarche in Finland was reported 14.88 years in *1943* (*Vara*) and 13.31 years in *1966* (*Metsälä*) Most of the lesions (in 67 cases or in 88 per cent) had been acquired during coitus. Four of the nine noncoital lesions were produced by the diagnostic introduction of an indwelling speculum, one by a therapeutic syringe applicator introduced into the vagina, and a sixth by the introduction of a man s fist into the vagina in the latter case a 10 cm longitudinal rupture had been produced into the left-hand wall of the vagina, and several ruptures were apparent in the mucous membrane of the vulva In the following three cases the vaginal rupture had been produced without forcible intervention inside the organ itself

Case 1 Age 44 years Married 22 years 2 children The patient had slipped in f ont of moving streetcar which hit and fractured her left hip In the left hand wall of the vagina rupture was found, beginning from the introitus

Case 2 77 year old widow 2 children and 1 abortion Menopause 44 years The patient had lost her balanc and had fallen on the ground The resulted

Table III *Localization of Ruptures in Vaginal Produced During Coitus*

Locality	Introitus	Lateral Wall	Vaginal Vault	Total
Number (per cent)	8 (14 %)	12 (21 %)	37 (65 %)	57

Table IV *Localization of Ruptures in the Vaginal Vault*

Locality	Left	Right	Posterior	Total
Number (per cent)	7 (19 %)	19 (51 %)	11 (30 %)	37

in haemorrhage Ruptures were found in both lateral fornices reaching the lower part of the vagina.

Case 3 57 year old childless divorcee who had 10 years earlier undergone a hysterectomy because of fibroids Lifting a heavy barrel produced a sharp pain in the lower abdomen and profuse bleeding. The introitus was found ruptured bilaterally

Fifty-five (82 per cent) of these patients were nulliparous (one had suffered an abortion) 6 (9 per cent) had delivered one child, and 6 (9 per cent) were multiparous with up to 7 deliveries.

The localization of the lesions had been recorded in 57 cases (Table III) The distribution by localization of ruptures in the vaginal vault is shown in Table IV

In our series ruptures produced during coital activity appeared mostly as 3–5 cm long lesions in the mucous membranes of the vagina. No perforations of the peritoneum or the rectum were observed. The ruptures are frequently accompanied by profuse bleeding In 36 per cent of the cases in our series blood transfusion of from 1 to 4 units was part of the treatment Two of the patients were brought into the hospital in a state of shock

Hospitalization of the patients varied from 1 to 14 days, with an average of 6 6 days 85 per cent of the cases were treated by suturing, mostly followed by antibiotic and or chemotherapeutic medication the remaining 15 per cent stayed in hospital for antibiotic treatment or for observation only Complications ensued in two cases a secondary wound infection developed in one patient and in the other a fistula was formed in the posterior lip of the cervix uteri No fatalities were recorded.

Discussion

Vaginal ruptures which require hospital treatment are obviously infrequent. Approximately 88 per cent were produced during coital activity and the rest mostly by other kinds of direct mechanical effects The three cases in which the lesions were produced without direct intravaginal intervention were all women of menopausal or postmenopausal age. It seems true, as has been stated by *Purnell* that a vaginal wall atrophied by ageing may be ruptured merely by a sudden increase in intraabdominal pressure.

Ruptures produced in association with coital activity appeared, on the other hand, mostly in women of fertile age. No marked infantilism could be attributed to this group as judged by their normal average age of menarche. A majority of the affected patients were unmarried and childless, but coital ruptures also appeared in married and multiparous women.

The phenomenon first observed in 1899 by *v Neugebauer* and later confirmed by others, that coital ruptures appear most frequently in the posterior part of the vaginal vault and in its right hand wall, is evident also in the present material. *v Neugebauer's* claim, that this reflects the woman's habit of moving her body forcibly to the left when defending herself has not gained support. *Richter* (1966) has shown a true picture of the living anatomy of the vagina by means of vaginal casts with silicon material. In general there is considerable asymmetry in the shape of the vagina, with the cervix mostly to the left of the midline. Thus, it could be assumed, when the penis intrudes into the vagina it commonly meets the right lateral fornix.

A more popular claim that coital ruptures are due to a spasm in the vaginal wall which would result from excessive sexual stimulation in the woman seems equally unlikely in the light of observations published by *Masters* and *Johnson*. These workers have studied more than 7,500 orgasmic cycles and state that following sufficient sexual stimulation, the vagina accomodates a penis of any size without difficulty" From their results it appears that even exceptionally small vaginas expand actively as a result of sexual stimulation, and no spasms appear in the upper two-thirds of the vaginal barrel even during orgasm. Thus it appears that the

direct opposite to earlier claims would be nearer to the truth, i.e. vaginal rupture seems a more likely complication of coital activity in women insufficiently stimulated than in those overexcited. This assumption is supported also by the finding of more frequent ruptures in unmarried patients who have been found by several authors to achieve orgasm less frequently than married women.

SUMMARY

After reviewing the factors assumed by previous writers to be causative in the production of vaginal ruptures the authors present their own series of 76 cases of such trauma which have been treated in hospital. On the basis of their own findings and of recently published data on the physical events in the vagina during coitus and on its living anatomy the authors conclude that despite earlier claims to the contrary insufficient sexual stimulation is a likely factor causing vaginal rupture during coitus while the localization of the trauma may be largely determined by the asymmetrical anatomy of the vaginal passage.

REFERENCES

Engel K. Zbl Gynäk 35 1189 1965
Maue H. Zbl. Gynäk. 25 1013 1959
Masters W H and *Johnson V E.* Human Sexual Response Little Brown and Co. Boston 1966
Metsälä P. Ann. Chl Gynaec Fenn 55 214 1966
v. Neugebauer F. Monatschr Geburtsh u. Gynäk 9 221 1899
Purnell L. W. J Obstet. Gynec Brit Commonw 72 799 1965
Rahm J. Duodecim 6 355 1925
Richter K. Geburtsh. u Frauenheilk. 9 1217 1966
Vara P. Über den Einfluss des zu verschiedenen Zeitpunkten Stattfindenden Spontanen Blasensprungs auf den Geburtslauf – Diss. Helsinki 1943

Received on Oct 6 1967

Ozaras, Husnü, Acta obst. et gynec. scandinav. 47 ... 1968

From the Department of Diagnostic Roentgenology (Associate Professor N. P. G. Edling, M.D.) and the Department of Women Diseases (Professor Ulf Borell) Karolinska Sjukhuset Stockholm Sweden

THE VALUE OF PLASTIC OPERATIONS ON THE FALLOPIAN TUBES IN THE TREATMENT OF FEMALE INFERTILITY

A Clinical and Radiological Study

BY

HÜSNÜ ÖZARAS

The treatment and causes of female infertility have been much discussed. According to the literature disorders of the Fallopian tubes account for 50 per cent of cases.

Operations on the tubes such as salpingolysis, salpingostomy resection of the tubes combined with salpingostomy and resection of the isthmic portion combined with implantation of the ampullary portion into the uterus have been extensively employed for the treatment of female infertility. The reported success rates for these operations vary greatly. *Westman* (1951) reported that of 453 patients who had a restorative operation on the tubes 90 or 19.8 per cent became pregnant, i.e. 6.4 per cent following salpingostomy 35.8 per cent following salpingolysis and 20 per cent following implantation of the tube into the uterus. The success rates given by *Mulligan, Rock* and *Easterday* (1953) for tubo-uterine implantation combined with polyethylene tubing and fimbrial repair were 10 per cent and 24 per cent respectively. *Green-Armitage* (1957) reported that of cases in which the patient became pregnant following implantation of the tube into the uterus combined with polyethylene tubing, 36.1 per cent carried their pregnancy to term and gave birth to a living child, in 5.2 per cent pregnancy terminated in miscarriage and 2.6 per cent had an extra-uterine pregnancy. *Moor-White* (1960) re-

ported that of the cases in which tubo-uterine implantation was performed the patient conceived in 56 per cent but only 31 per cent carried their pregnancy to term and gave birth to a living child. *Pulgmacia* (1960) reviewed the results of 3018 plastic operations performed by experienced and skilled gynaecologists, and found that pregnancy resulted in 22.3 per cent being intra uterine in 19 6 per cent and tubal in 2 7 per cent. These workers considered salpingolysis to be the most successful procedure resulting in pregnancy in 35 1 per cent of cases tubo-uterine implantation with a success rate of 28 per cent coming next. *Palmer* (1964) reported that of 489 tubo-plastic operations which he had performed in the years from 1949 to 1961 21 per cent were followed by intra uterine and 6 per cent by extra-uterine pregnancy The success rates for the different types of operation were as follows tubo-uterine implantation 33 per cent salpingolysis 28 per cent cuff" salpingostomy 29 per cent gutter salpingostomy on the ampullary portion of the tube 10 6 per cent salpingostomy on the isthmic portion of the tube and implantation of the ovary into the uterus were unsuccessful *Swolin* (1967) refined the technique used in plastic operations on the tube and reported that intra-uterine pregnancy followed in 28 per cent of the patients in his series who were operated upon by this method.

A fair assessment of the therapeutic value of the above operations presents difficulties for the following reasons One reason is the discrepancy between the reported results. A further reason is that the gross and microscopic appearances of the specimens removed at operation have been rarely described. Moreover in cases in which unilateral tubal repair was carried out, information about the condition of the contralateral tube is usually lacking. Success may therefore have been credited to the operation in cases in which one tube was healthy prior to the operation.

Thus the therapeutic effect of the individual plastic operations on the tubes cannot be properly assessed unless there is positive proof that both tubes were occluded prior to the operation.

Hystero-salpingography is the best method for demonstrating abnormalities of the tubes such as dilatation, constriction or closure of the lumen thickening or atrophy of the tubal mucosa and failure of peritoneal spill.

During the past twenty-five years a large number of women attended the Department of Women's Diseases of this hospital complaining of infertility and were treated by surgery on the tubes following hystero-salpingography. This prompted the present investigation in which the hystero-salpingograms and the hospital records of these patients were reviewed with special reference to the radiographic findings, the gross and microscopic appearances of the operation specimens and the operative results. In studying the hystero-salpingograms special attention was paid to a possible relationship between certain radiographic features of the tube and the operative results.

Case Material and Methods

During the years from 1940 to 1964, 600 women attended the Department of Women's Diseases of this hospital for infertility. All these patients were treated by surgery, the majority of the operations being performed in the years from 1941 to 1950. Most of these cases have been previously reported by *Westman* (1951). A review of the hospital records and the findings at the follow-up examination of these patients afforded complete information in 300 patients. These latter cases are now discussed in more detail.

Hystero-salpingography was done on 288 patients; in the other 12 patients the records on the gross and microscopic appearances afforded satisfactory information on the pre-operative condition of the tube. It was therefore considered justifiable to include these cases in the present investigation.

Before 1948 a water soluble and thereafter different types of viscous water soluble media were used for hystero-salpingography. In order to obtain films of the highest possible quality the views were drastically coned, using about 70 kV and a moving grid. One hundred and fifty two of the 300 patients were operated upon unilaterally and 148 bilaterally. The types of operations performed are shown in Tables I and II. Infertility was primary in 201 and secondary in 99 patients.

Cases in which pregnancy followed the operation

Of the 300 patients 31 or 10.3 per cent became pregnant after the operation; 17 went to term and gave birth to a living child

Table I *Unilateral Surgery on the Fallopian Tubes*

Operative Results	Salpingolysis	Resection of Tube and Salpingostomy	Resection of Tube and Salpingostomy + Salpingolysis	Tubo-uterine Implantation	Ovario-uterine Implantation
Birth of living child	4	1	3	1	—
Abortion	1	1	1	2	—
Extra-uterine pregnancy	1	1	—	1	—
No pregnancy	14	70*	29	21	1
Total	20	73	33	25	1

Cholesterol-oleate plug
Cholesterol-oleate plug, 39 cases
Polyethylene tubing one case
Polyethylene tubing, 2 cases

in 9 patients pregnancy terminated in miscarriage and 5 patients had an extra-uterine pregnancy Table III shows the time interval between the operation and conception Infertility was primary in 19 and secondary in 12 patients in this group

The ages of the patients at the time of the operation ranged from 28 to 36 years and the interval before conception varied from 1 to 5 years (Table III) None of these patients had a history of gonorrhoea or tuberculous salpingitis.

On the basis of the radiographic findings and the gross and microscopic appearances of the specimens removed at operation (as described in the hospital records) these 31 cases were divided into the following groups

Group I 9 cases in which one tube was operated upon the contra lateral tube showing no abnormality Pregnancy with the birth of a living child followed the operation in 2 cases in 4 cases pregnancy terminated in miscarriage and 3 patients had an extra-uterine pregnancy

Group II 12 cases in which the tube was macroscopically and radiographically normal There was unilateral or bilateral spill and

Table II *Bilateral Surgery on the Fallopi Tubes*

Operative Results	Salpingolysis	Resection of Tube and Salpingostomy	Tubo-uterine Implantation	Implantation of One Tube into Uterus + Salpingolysis on Contralateral Tube	Implantation of One Tube into Uterus + Resection of Contralateral Tube and Salpingostomy	Resection of One Tube Combined with Salpingostomy and Cholesterol-oleate Plug + Salpingolysis on Contralateral Tube	Ovario-uterine Implantation + Resection of Contralateral Tube and Salpingostomy
Birth of living child	3	1	3	1	—	—	—
Abortion	—	2	—	1	—	1	—
Extra-uterine pregnancy	—	2	—	—	—	—	—
No pregnancy	11	101*	7	8	6	1	1
Total	14	106	10	10	6	2	1

Cholesterol-oleate plug, one case
Cholesterol-oleate plug, 47 cases
Salpingolysis, 18 cases

Table III *Time Interval between Operation and Conception*

Time Interval in Years	No. of Cases in which Patient conceived
1	14
2	8
3	—
4	3
5	[illegible]
6	3
7	1

salpingolysis vas carried out. Thus one or both tubes might have been *normal in these cases In 9 cases the patient gave birth to* a living child and in 3 cases pregnancy terminated in miscarriage

Group III 10 cases in which both tubes showed abnormalities and pregnancy followed surgery on the tubes. Six of these patients carried their pregnancy to term and gave birth to a living child in 2 cases pregnancy terminated in miscarriage and 2 patients had an extrauterine pregnancy

In group III 8 patients underwent bilateral surgery for abnormalities of the tubes (confirmed by pre-operative hysterosalpingography and/or histology of the operation specimens) and became pregnant following the operation. In the other 2 cases one tube was so severely damaged that an operation would have been of *no avail. For this reason only unilateral surgery was performed.* Two of these patients had an extra-uterine and the other 8 an intra-uterine pregnancy These latter cases are now discussed in more detail.

Infertility was primary in 5 cases and secondary in 3 The following operations were performed in this group *bilateral salp*ingolysis, 2 cases unilateral resection of the tube, one case bilateral resection of the tubes 2 cases unilateral tubo-uterine implantation, one case and bilateral tubo-uterine implantation, 2 cases.

As the operative technique varied greatly over the years and the operations were performed by different surgeons it was considered to be of interest, to study the years in which these 8

patients underwent surgery It was found that they were not operated upon during a particular 5-year period of the 25-year period of study the success rates for the individual 5-year periods being virtually the same.

The radiographic findings in these 8 cases were as follows In 2 cases both tubes failed to fill in one case, only the intramural portion of both tubes filled in 2 cases the ampullary portion of both was 1 cm in diameter there was no peritoneal spill and the relief of the tubal mucosa did not show any abnormality bilaterally in one case the ampullary portion of both tubes was 1.5 cm in diameter there was no peritoneal spill bilaterally the mucosal relief of both tubes did not show any abnormality In one case the tubal diameter was 1 cm, that of the contra-lateral tube being 2 cm there was only unilateral peritoneal spill and the mucosal relief of both tubes did not show any abnormality in one case both tubes were 1 cm in width and the mucosal relief did not show any abnormality bilaterally but peritoneal spill failed to occur on both sides.

Cases in which pregnancy did not follow the operation

Of the 300 cases in this series the operation was unsuccessful in 269 Tables I and II show the types of operation performed on these patients Infertility was primary in 182 and secondary in 87 cases Tubal surgery was performed unilaterally on 135 patients and bilaterally on 134 The ages of the patients ranged between 21 and 42 years the majority being between 26 and 37 years at the time of the operation. Twenty-six of the 269 patients had a history of gonorrhoea and 10 of tuberculous salpingitis.

On the basis of the radiographic findings the 269 cases were divided into the following groups

Group I No peritoneal spill	*Total* 150 cases
No filling or incomplete filling of both tubes	29 cases
Bilateral filling of normal tubes,	21 cases

normal" applies to tubes whose ampullary portion was 1 cm or less in diameter (as measured directly on the film) and whose mucosal fold did not show any abnormality

Unilateral filling of normal tube	3 cases
Unilateral filling of dilated tube	21 cases
Bilateral filling, one tube being dilated,	13 cases
Bilateral filling of dilated tubes	63 cases
Group II Unilateral peritoneal spill	Total 63 cases
Bilateral filling of normal tubes	4 cases
Unilateral filling of normal tube	5 cases
Unilateral filling of dilated tube	11 cases
Bilateral filling one tube dilated	22 cases
Bilateral filling both tubes dilated,	21 cases
Group III Bilateral peritoneal spill	Total 45 cases
Normal tubes	9 cases
One tube dilated	9 cases
Both tubes dilated	27 cases

Discussion

Of the 300 patients in this series 31 or 10.3 per cent became pregnant following surgery on the tube 8 6 per cent had an intra uterine and 1 7 per cent an extra-uterine pregnancy the majority conceived within two years of the operation These success rates tally largely with those published in the literature but a critical review of the hysterosalpingograms of these patients and the histological appearances of the operation specimens showed that success was not unquestionably due to the operation in quite a number of these 31 cases

In 9 cases in which one tube was apparently normal and was not operated upon, it is open to some doubt whether success can be credited to the operation performed on the contra lateral tube. It is more likely that the normal tube was responsible for the subsequent pregnancy

In 12 cases pre-operative hystero-salpingography had shown unilateral peritoneal spill. This suggests that one or both tubes might have been functioning normally prior to salpingolysis It is

dilated" applies to tubes whose ampullary portion was more than 1 cm in diameter



Fig. 1 Hystero-salpingogram taken prior to surgery on the tubes on a patient who became pregnant following the operation.

difficult to say whether the operation or pre-operative hystero-salpingography were of therapeutic value in these cases

In 10 cases there was evidence of abnormalities of both tubes and surgery was carried out. There is therefore little doubt that success was due to the operation in these cases. However 2 of these patients had an extra-uterine pregnancy. This suggests that the tubes functioned normally only in 8 of these 10 patients. Thus the operation was unquestionably successful in only 8 or 2.7 per cent of the 300 patients in this series. This success rate is considerably lower than that published in the literature. However the number of successful cases is too small to permit a statistical analysis of the results to be made. It is interesting to note that some of these patients were 35 years old. This suggests that a plastic operation on the tubes should be given a trial even in older patients of reproductive age who seek medical advice for infertility. It should also be remembered that the interval between the operation and conception may be as long as five years.

Characteristic features of the hystero-salpingograms of the 8 patients who became pregnant following surgery on the tubes were (i) that both tubes were either apparently normal or only slightly dilated, (ii) that the mucosal relief of both tubes did not show any abnormality (Fig. 1) and (iii) that there was no

Unilateral filling of normal tube	3 cases
Unilateral filling of dilated tube	21 cases
Bilateral filling, one tube being dilated,	13 cases
Bilateral filling of dilated tubes,	63 cases
Group II Unilateral peritoneal spill	*Total* 63 cases
Bilateral filling of normal tubes	4 cases
Unilateral filling of normal tube	5 cases
Unilateral filling of dilated tube	11 cases
Bilateral filling one tube dilated	22 cases
Bilateral filling both tubes dilated,	21 cases
Group III Bilateral peritoneal spill	*Total* 45 cases
Normal tubes	9 cases
One tube dilated,	9 cases
Both tubes dilated	27 cases

Discussion

Of the 300 patients in this series 31 or 10.3 per cent became pregnant following surgery on the tube 8 6 per cent had an intra uterine and 1 7 per cent an extra-uterine pregnancy the majority conceived within two years of the operation. These success rates tally largely with those published in the literature but a critical review of the hysterosalpingograms of these patients and the histological appearances of the operation specimens showed that success was not unquestionably due to the operation in quite a number of these 31 cases.

In 9 cases in which one tube was apparently normal and was not operated upon it is open to some doubt whether success can be credited to the operation performed on the contra-lateral tube. It is more likely that the normal tube was responsible for the subsequent pregnancy

In 12 cases pre-operative hystero-salpingography had shown unilateral peritoneal spill This suggests that one or both tubes might have been functioning normally prior to salpingolysis It is

dilated" applies to tubes whose ampullary portion was more than 1 cm in diameter

results which he achieved, were unquestionably due to his experience and skill. It is to be hoped that with further improvement of the operative technique better results will be achieved. Careful screening of the cases of infertility to be treated by surgery will also contribute to improving the results of operation.

SUMMARY

Out of the 300 patients in this series on whom a plastic operation on the tube was carried out in the Department of Women's Diseases of the Karolinska Sjukhuset in Stockholm, 10.3 per cent conceived following the operation. However a critical review of the hystero-salpingograms and hospital records of these patients showed that success was unquestionably due to the operation only in 2.7 per cent

Surgery was successful only in the cases in which there was radiographic evidence of a normal tubal mucosa and of tubes which were either normal in width or slightly dilated.

The view is expressed that with careful screening of the cases of infertility better results will be achieved.

The view was confirmed that a plastic operation on the tube is of no avail in patients with a history of gonorrhoea or tuberculous salpingitis.

REFERENCES

Barnett E J Fac Radiol 7 115 1955

Castillo Mario A and *Weiner Amos S* Hawaii MJ 23 285 1964

Ernst Amalia and *Bravo Abraham* Bol Soc Chilena Obst y Ginec 23 198 1958

French M J New England Med J 241 686 1949

Geller, J and Mueller F Arq Brasil Ginec. 42 61 1956

Gillespie Henry W Brit J Radiol 38 448 301 1965

Green-Armytage V B J Obst & Gynaec. Brit Emp 64 47 1957

Hayashi Motoyuki Am J Obst & Gynec. 84 79 1962

Hellman Lend M J Obst & Gynaec Brit Emp. 63 852, 1956

Moore-White Margaret Internat J Fertil 5 237 1960



Fig 2. Hystero-salpingogram taken prior to surgery on the tubes on a patient who failed to conceive following the operation. Both tubes are slightly dilated, the mucosal folds are not seen and there is no peritoneal spill.

peritoneal spill in the majority. In the cases in which tubal repair was unsuccessful, there was pre-operative evidence of thickening or absence of the tubal mucosa and markedly dilated tubes in more than 50 per cent (Fig 2). There appears to be a relationship between these radiographic findings and the operative results. This substantiates the value of hystero-salpingography in selecting the patients to be treated by surgery.

In the present series the best results were achieved with salpingolysis and tubo-uterine implantation but the number of cases in which these operations were unquestionably successful was too small to permit any definite conclusions to be drawn about which of these prodecures is the most successful.

The results of tubal repair were less encouraging than has previously been assumed, but it should be remembered that the operations were performed by different more or less experienced surgeons and that the operative technique varied greatly over the years. The relationship between the operative technique and the results has been demonstrated by *Swolin* (1967). The excellent

Grönroos M., Laakso, L. Rauramo, L. and Aalto, T. Acta obst. et gynec. scandinav. 47 [illegible], 1968
From the Department of Obstetrics and Gynaecology (Professor Lauri Rauramo) the Department of Roentgenology (Professor Pekka Virtama) and the Central Laboratory (Ben Laurent M.D.) University Central Hospital Turku Finland

THE VALUE OF LYMPHOSCINTIGRAPHY IN EVALUATING THE EXTENT OF MALIGNANT TUMOURS IN THE FEMALE PELVIS

BY

M. GRÖNROOS, L. LAAKSO, L. RAURAMO AND T. AALTO

According to the Cancer Committee of the International Federation of Gynecology and Obstetrics (1961) the clinical staging of cancer of the uterus and vagina should be based on careful clinical examination prior to therapy supplemented, in order to clarify the nature and spread of the malignant tumour by various diagnostic methods such as colposcopy curettage, conisation or amputation of the cervix, and simple X-ray examinations of the skeleton and lungs Although the above investigations throw only limited light especially on the extent of soft tissue invasion in the pelvis and abdomen no other diagnostic methods to aid with the clinical classification of these malignant conditions have been widely accepted (*Kottmeier* 1967)

Yet accurate knowledge of the anatomical spread is of vital importance in the treatment of a malignant tumour Certain radiographic techniques urography arteriography venography and lympography help to fill this diagnostic gap alongwith various gynaecological punctures and laparoscopy However all these methods have drawbacks they may require hospitalisation the technique or interpretation of the results may be difficult the examinations may be time-consuming or involve risk to the patient For these reasons it is still important to evolve new methods

Mulligan William J *Rock John* and *Easterday Charles L* Fertil. Steril. *4* 428 1953
Palmer R. Personal communications in Vienna, May 1964
Pidgmacla L. Pous. Rev Espan. Obst. y Ginec. *114* 3 1960
Sherman Albert J. A. M. A. *148* 603 1952
Siegler Alvin M and *Hellman Louis M* Fertil Steril. *7* 170, 1956
Swolin Kurt Acta obst. et gynec. scandinav *46* Suppl. *4* 1967
Westman Axel Acta obst. et gynec. scandinav *30* 186 1951
Weir William C *Weir David R.* and *Littel Arthur S* Am. J Obst. & Gynec. *73* 412, 1957
Winson S Am. J Obst. & Gynec. *52* 631 1946

Received on Sept. 12, 1967

carcinomas were managed with radium packing method, followed if possible by operation and, when necessary telecobalt therapy Recurrences were treated with percutaneous cobalt or sometimes, intravaginal radium.

Lsg was performed on three patients with cervical cancer (Nos. 1 9 and 18) by injecting 50 μCi of colloidal Au^{198} into each lateral fornix. The examination was carried out prior to therapy The scannings were performed on each patient at intervals of 3-8 hours after the injection for 48 hours.

Results

Table I shows that the lymphoscintigraphic examination was performed at least once on 58 patients and that it *failed* in two cases (Nos. 42 and 57) and had a partial failure in one case (No. 19) The reason for the failure in these two cases was that the patient moved because of severe pain from pelvic metastases. When the pain was eliminated by suitable premedication, a repeat examination of one of these two patients one week later was successful. The poor condition of the other patient prevented a repeat examination. The reason for the partial failure with the third patient is not known. In this case a repeat examination carried out three months later was perfectly satisfactory

To obtain as reliable an idea as possible of the anatomical distribution of the disease the observations obtained from clinical, urographic and lymphographic studies at the time of lymphoscintigraphy were followed up six months later Tables I and III show that *the final diagnosis arrived at in this manner agreed* with the lsg finding in 49 cases (86 per cent) A false negative result was obtained by lsg in three patients (Nos 18, 22 and 56 Fig. 1) i.e 5 per cent, and a false positive finding in five patients (Nos 7 10 14 34 and 35) i.e 9 per cent. In cases 18 and 56 in which the lsg result was negative the lymphographic appearance was suspicious although the examination was successful only on one side The former patient was found six months later to have lymphatic stasis in the lower limb and infiltration of the ipsilateral parametrium, and latter patient developed a metastasis in the supraclavicular fossa. Distant metastases and parametrial

Lymphoscintigraphy has been recommended as a technically easy method which is painless and not harmful for the patient who may be ambulant (e g *Lang*, 1960 *zum Winkel* and *v Keiser* 1964 *Voutilainen* and *Wiljasalo* 1965) The purpose of the present study was (1) to analyse the value of the method in estimating the extent of gynaecological cancer the involvement of nodes and presence of pelvic and abdominal metastases (2) to study its applicability as a projector of the uterine lymph vessels and glands and (3) to examine the effect of radiotherapy on the lymph vessel system of the pelvis

Material and Methods

Routine lymphoscintigraphy (=lsg) was performed on a total of 58 patients by injecting 100 μCi of colloidal Au^{198} subcutaneously into the dorsum of each foot. The technique has been explained thoroughly in earlier studies e g *Voutilainen* and *Wiljasalo* (1965) Eighteen to 20 hours was the time established empirically by the authors as the most suitable interval between the injection and the investigation. All the patients had some gynaecological disease the overwhelming majority a malignant tumour or its recurrence The gynaecological diagnoses and the clinical staging of carcinoma of the cervix, corpus and ovaries are given in Table I

It was possible in 57 cases to compare the lsg finding with the clinical finding, that is the result of all the examinations that can be used in accordance with international practice as the basis of clinical staging The result was compared also with the lymphography finding in 18 cases and intravenous urography performed in 44 cases. The results of the comparative studies mentioned were supplemented by a six month follow up to achieve the greatest possible reliability

The pre treatment lsg finding was compared in 14 patients with the finding of a repeat examination 3–6 months after radiotherapy (Table II) These patients had primary or recurrent carcinoma of the cervix or of the corpus

Patients with carcinoma of the cervix were treated with intracavitary radium and telecobalt to the parametria, and corpus

	—	—		±	(—)
Ca cervicis ut gr IIb radiata		f		+	(+)
(recurrent)				±	(+)
Ca cervicis ut gr IIb radiata Metast. apicum		f	±		(+)
C cervicis ut gr III			—	+	(+)
		ff	—	±	(—)
adiata			±	+	(+)
(recurrent)	±		±	+	(+)
(recurrent)	±	ff	+	+	(+)
persist		ff	+	+	(+)
persist		±	—	±	(+)
	±		—	+	(+)
	+			+	(+)
Ca cervicis ut gr IV	±		+	+	(+)
			—	—	(—)
Ca in situ cervicis ut. oper	±	—		—	(—)
Dysplasia cervicis ut.	±	—		—	(—)
Ca corporis ut gr I	—	f ±	±	±	(+)
Ca corporis ut. gr II	+			—	(—)
	—			—	(—)
	—		—	±	(—)
radiata	—		—	±	(+)
radiata	+		—	+	(+)
(recurrent)	ff				
Ca corp. ut. gr II radiata et operata recidivans	+	+	+	+	(+)
Ca corp ut gr II adiata et operata recidivans				+	(+)
	+	+		+	(+)
Ca corp. ut. gr III operata (recurrent)	+				

Table I *Lymphoscintigraphy Findings and Their Correlation with Clinical Examination and Lymphangiography or Urography*

(+ = pathological finding, ± = suspect — = normal ff = failure f = partial failure)

Patient No.	Diagnosis		Lymphoscintigraphy	Lymphangiography	Iv Urography	Clinical Finding
1	Ca cervicis ut gr I a		—	—	—	— (—)
2			—		—	— (—)
3			—	—	±	— (—)
4			—		—	— (—)
5			—		—	— (—)
6		(treated surgically)	—		—	— (—)
7	Ca cervicis ut gr I b		±	ff	—	— (—)
8	~		—	ff	—	— (—)
9			—	—	—	— (—)
10		(treated with radiother.)	±	ff		± (—)
11		(treated with radiother.)	—		—	± (—)
12		(recurrent)	+	+	+	+ (+)
13		radiata Metast. vaginae	—		—	± (—)
14		II a radiata	±		—	± (—)
13		(recurrent)	+		+	+ (+)
16		(recurrent)	+	ff	—	+ (+)
17		(recurrent)	±	—	+	+ (+)
18	Ca cervicis ut. gr II b		—	f ±	—	— (+)
19a		radiata	f ±	f ±	—	± (+)
19b		(recurrent)	+		+	+ (+) (3 months later)

Ca cervici ut gr IIb	radiata	—	—		±	(—)
	(recurrent)		f		+	( )
Ca cervicis ut gr IIb	radi ter Metast vaginae		±	—	±	(+)
C cervicis ut gr III			f	±	+	(+)
				—		(+)
	radiata	—	ff	—	±	(—)
	(recurrent)	±		±	+	(+)
	(recurrent)	±		±	+	(+)
	perust	+	ff	+		(+)
	perust		ff	+	+	(+)
		±	±	—	±	(+)
Ca cervicis ut gr IV		+		—	+	(+)
		±			+	(+)
		+		+	+	(+)
Ca in situ cervicis ut. oper		±	—	—	—	(—)
Dysplasia epith. cervicis ut.		±	—		—	(—)
Ca corporis ut. gr I		—	f±		—	(—)
Ca corpori ut. gr II		+		±	±	(+)
		—			—	(—)
		—			—	(—)
	radiata	—		—	±	(—)
	radiata	+		—	±	(+)
	(recurrent)	ff		—	+	(+)
Ca corp. ut gr II radiata et operata recidivans		+	+	+	+	(+)
Ca corp ut. gr II radiata et operata recidivans		+	+		+	(+)
Ca corp. ut. gr III operata (recurrent)		+			+	(+)

: 1. Contin.

nt	Diagnosis	Lymphoscintigraphy	Lymphangiography	Iv Urography	Clinical Finding
	Ca corp ut. gr III radiata (ecurrent)	±			+ (+)
	Ca ovar gr I a operata (recurrent)	±		—	+ (+)
	Ca ovar gr I b oper et radiata	—		—	— (—)
	Ca ovar gr I b oper et radiata	—	—	—	— (—)
	Ca ovar gr II a	—		—	— (—)
	Ca ovar gr III	+		—	+ (+)
	Ca ovar gr IV oper et radiata	±			+ (+)
	Tumor malignum pelvis	+			+ (+)
	Pyosalpinx l sin	—			— (—)
	Sactosalpinx l a	—			— (—)
	Ca peritonei Pseudomyxoma append.	—	f ±	±	— (+)
	Ca vulvae metastaticum	ff			+
		+			+ (+) (1 week later)
	Myosarcoma vaginae	—		—	— (—)

Clinical examination includes all the diagnostic aids which can be used according to the international rules in clinical staging (see ntroduction)

— = no suspicion of spread outside the genitals or recurrence ± = uspicious and + = verified spread or recurrence ff = failure f = rtial failure the corresponding signs in brackets refer to the final i.e overall diagnosis made after a six month follow-up period.

Table II. *The Effect of Radiotherapy on Lymphatics Evaluated by lsg*

Patient No	Lymphography Performed Before Therapy (− +)	Uptake in Control lsg After Radiotherapy
1	−	− −
2	−	−
3		− −
7	−	− −
10	−	− −
12		− −
13	−	−
18	+	=
22	−	− −
25	−	±
30	+	− −
35		+
36	+	− −
44		− −

− − uptake decreased appreciably compared with the preceding examination

− uptake decreased slightly

± uptake equal to earlier

uptake more pronounced than earlier

Table III. *Comparison of Lymphoscintigraphy, Clinical Examination, Lymphography and Intravenous Urography with the Final Diagnosis Made after a Six Month Follow-Up Period*

	Lsg	Clinical Examination	Lymphography	Iv Urography
Total	57	57	18	44
False negative				
per cent	5	4	11	25
False positive				
per cent	9	12	6	2
Agreement				
per cent	86	84	83	73

…le I. Contin.

…ient	Diagnosis	Lymphoscintigraphy	Lymphangiography	Iv Urography	Clinical Finding
	Ca corp ut. gr III radiata (recurrent)	±			+ (+)
	Ca ovar gr I a operata (recurrent)	±		—	+ (+)
	Ca ovar gr I b oper et radiata	—		—	— (—)
	Ca ovar gr I b oper et radiata	—	—	—	— (—)
	Ca ovar gr II a	—		—	— (—)
	Ca ovar gr III	+		—	+ (+)
	Ca ovar gr IV oper et radiata	±			+ (+)
	Tumor malignum pelvis	+			+ (+)
	Pyosalpinx l sin	—			— (—)
	Sactosalpinx l a	—			— (—)
	Ca peritonei Pseudomyxoma append.	—	f ±	±	— (+)
…a	Ca vulvae metastaticum	ff			+
…b		+			+ (+) (1 week later)
	Myosarcoma vaginae	—		—	— (—)

Clinical examination includes all the diagnostic aids which can be used according to the international rules in clinical staging (see "Introduction")

— = no suspicion of spread outside the genitals or recurrence ± = suspicious and + = verified spread or recurrence ff = failure f = partial failure the corresponding signs in brackets refer to the final i.e. overall diagnosis made after a six month follow-up period.

Table II. *The Effect of Radiotherapy on Lymphatics Evaluated by Lsg*

Patient No.	Lymphography Performed Before Therapy (– +)	Uptake in Control Lsg After Radiotherapy
1	–	– –
2	–	–
3	+	– –
7	–	– –
10	–	– –
12		– –
13	–	–
18	+	±
22	–	– –
25	–	±
30	+	– –
35		+
36		– –
44		– –

– – uptake decreased appreciably compared with the preceding examination

– uptake decreased slightly

± uptake equal to earlier

uptake more pronounced than earlier

Table III. *Comparison of Lymphoscintigraphy, Clinical Examination, Lymphography and Intravenous Urography with the Final Diagnosis Made after Six Month Follow-Up Period*

	Lsg	Clinical Examination	Lymphography	Iv Urography
Total	57	57	18	44
False negative per cent	5	4	11	25
False positive per cent	9	12	6	2
Agreement per cent	86	84	83	73



a



b

Fig. 1. Patient No. 56: by lymph graphy metastatic glands are shown on the right side but not clearly demonstrated by lymphoscintigraphy



b

Fig. 2 Patient No. 12, metastatic process verified both lymphoscinti- and lymphographically

infiltration in addition to a vaginal recurrence were revealed later also in patient No 22 On the other hand, the five false positive cases in which lsg was slightly suspicious each proved to have no lymphatic spread of the disease

We also compared *the findings on the initial clinical examination* with the final diagnosis in 57 cases Agreement was found in 48 of these (84 per cent) A false positive clinical observation was made in seven cases (Nos 10 11 13 14 20 25 and 40) *i e* 12 per cent In all these cases there was only a slight suspicion of the spread of the disease beyond the genitalia The *clinical examination* gave *a false negative result in two cases* (Nos 18 and 56) *i e* in 4 per cent.

The lymphographic results were compared with the final diagnosis and lymphoscintigraphic findings in 18 patients. Lymphography failed technically in seven cases Even in these 18 patients the examination was successful in six cases on one side only Lymphography and lsg agreed in 10 cases *i e* 56 per cent (Fig 2) *These lymphographic observations also proved correct on comparison* with the other results. In the other eight cases the lymphography and lsg findings were conflicting the lymphography finding was correct in five of these patients (Nos. 18 22, 34 35 and 56) and the lsg finding in three patients (Nos 17 21 and 36) A false negative result was obtained *lymphographically* in two cases (Nos 17 and 21) and a false positive result was recorded for No 36 (Tables I and III)

Comparison of intravenous *urography* reports with the final diagnosis was possible in 44 and with the lsg findings in 43 cases. In the former a positive correlation was established in 32 cases, 73 per cent and in the latter in 30 cases 70 per cent. There was discrepancy between intravenous urography *and lsg finding* in 13 cases the lsg finding was correct in nine of them (Nos 3 16 19a 24 30 31 41 47 and 51) and the intravenous urography finding in four (7 14 34 and 56) A false negative result was obtained by intravenous urography in 11 cases (Nos 16 18 19a 22 24 30 31 41 42 47 and 51) *i e* in 25 cent *and a false* positive finding in patient No 3 (about 2 per cent)

The suitability of lsg for studying the *anatomy of the uterine lymphatics* and the spread of the malignant process into them

was evaluated also For this purpose the isotope solution was injected into each lateral fornix of three patients who had carcinoma of the cervix ranging from grade Ia to IIb A pre treatment examination showed that the radioactivity was distributed evenly throughout the uterus and its immediate vicinity and the different lymphatics or lymph glands could not be distinguished from one another

An attempt was also made by lsg, to establish *the effect of radiotherapy on the lymphatics and lymph glands.* Repeat lsg was performed on 14 patients 3–6 months after radiotherapy (Table II) This showed nine cases in which the uptake of the isotope was distinctly poorer than before treatment (Fig. 3) The uptake was slightly less in two cases No change was established in two patients In these both the uptake and the pattern of the lymphatics and lymph glands were identical with the earlier picture. Repeat lsg on a patient treated by total hysterectomy for dysplasia of the cervix (No. 35) and who thus received no radiotherapy at all, displayed more intense uptake than previously

Discussion and Conclusions

Our investigations substantiate earlier studies (*Hultborn et al.* 1955 *Lang*, 1960 *Gert* and *Delouche* 1963 *zum Winkel* and *von Keiser* 1964 *Voutilainen* and *Wiljasalo* 1965) which suggested that lsg is a safe and fairly rapid method of examination easily performed on an out-patient. Admittedly swelling, mild pain and rednes were seen in the feet of three patients after lsg. The condition subsided in a few weeks with the help of anti allergic drugs The examination failed on three patients, with two of them because the patient moved on account of pain caused by cancer metastases The reason for the partial failure of the third examination is not known but a repeat examination proved completely satisfactory

W[illegible] consider lsg to be a fairly reliable method, although perhaps not as accurate as lymphography Early spread by lymphatic [illegible] particularly difficult to detect by lsg (Fig. 1) In all the three [illegible]ases in which lsg gave a false negative finding, the lymphographic pictur[illegible] was slightly suspicious and later observation con-

infiltration in addition to a vaginal recurrence were revealed later also in patient No 22. On the other hand, the five false positive cases in which lsg was slightly suspicious each proved to have no lymphatic spread of the disease

We also compared *the findings on the initial clinical examination* with the final diagnosis in 57 cases Agreement was found in 48 of these (84 per cent) A false positive clinical observation was made in seven cases (Nos 10 11 13 14 20 25 and 40) i.e 12 per cent. In all these cases there was only a slight suspicion of the spread of the disease beyond the genitalia. The clinical examination gave a false negative result in two cases (Nos 18 and 56) i e in 4 per cent.

The lymphographic results were compared with the final diagnosis *and lymphoscintigraphic findings in 18 patients*. Lymphography failed technically in seven cases Even in these 18 patients, the examination was successful in six cases on one side only Lymphography and lsg agreed in 10 cases i e 56 per cent (Fig 2) These lymphographic observations also proved correct on comparison with the other results In the other eight cases the lymphography and lsg findings were conflicting the lymphography finding was correct in five of these patients (Nos 18 22, 34 35 and 56) and the lsg finding in three patients (Nos. 17 21 and 36) A false negative result was obtained lymphographically in two cases (Nos. 17 and 21) and a false positive result was recorded for No 36 (Tables I and III)

Comparison of intravenous *urography* reports with the final diagnosis was possible in 44 and with the lsg findings in 43 cases. In the former a positive correlation was established in 32 cases 73 per cent and in the latter in 30 cases 70 per cent. There was discrepancy between intravenous urography and lsg finding in 13 cases the lsg finding was correct in nine of them (Nos. 3 16 19a 24 30 31 41 47 and 51) and the intravenous urography finding in four (7 14 34 and 56) A false negative result was obtained by intravenous urography in 11 cases (Nos 16 18 19a 22 24 30 31 41 42 47 and 51) i e in 25 cent and a false positive finding in patient No 3 (about 2 per cent)

The suitability of lsg for studying the *anatomy of the uterine lymphatics* and the spread of the malignant process into them



c

Fig. 3 Patient No. 1 no suspicion about metastatic process both in lympho- and lymphoscintigraphy. Note the poorer uptake in the latter leg taken after irradiation

firmed the dissemination. Nor did intravenous urography reveal more than a slight abnormality in one of the three cases mentioned above. A false positive diagnosis (in each case no more than a slight suspicion) was made in five cases. However, an error of even this magnitude justifies the suggestion that radiotherapy or operative treatment should not be prescribed on the basis of lsg alone. We are of the opinion that all suspicious and perhaps even definitely abnormal findings revealed by lsg must be confirmed in some other way, *e.g.* lymphographically, although it must be remembered that a negative finding recorded by this latter method does not either exclude the possibility of microscopic spread (Eiken and Madsen 1965). Moreover microscopic changes have been observed in the lymphatics and blood vessels after lymphography performed with Lipiodol and, consequently



a



b

of the uterine cervix. These nodes were not visualised in our studies in scintigrams of the uterine cervix using colloidal Au[198] The calibre of the lymph vessels is obviously so small in this area that they are incapable of taking up the tracer that was injected in the present study

Migration of the tracer to the other side was seen in some cases (Fig. 4) These collateral channels may cross one another in some cases A similar phenomenon was observed by *Hreshchyshyn* and *Sheehan* (1965) after lymphangiography with Ethiodol.

Increased uptake of the tracer after telecobalt therapy was established by *zum Winkel* and *Scheer* (1965) in one case. We had opposite results (Fig. 3) The difference may be because in our study the patients were given both radium and telecobalt therapy to the lower abdomen, resulting in a greater post radiation effect.

SUMMARY

The alue of lymphoscintigraphy (=lsg) as a diagnostic method was studied on 58 gynaecological patients the overwhelming majority of whom had gynaecological cancer Lsg proved to be a usef l examination for estimating the invol ement of nodes and the presence of pel ic and abdominal metastases. The facility of the examination for both the investigator and the patient, its fairly rapid performance and freedom from risk permit its use on an outpatient basis Oedema and slight redness in the feet—obviously an allergic reaction—were a mild complication in three patients for 2–4 weeks after the examination.

Lsg is also a relatively reliable examination It equalled lymphography and clinical examination in diagnostic accuracy and gave a correct result in 86 per cent when compared with the final diagnos based on all the methods used and confirmed after a six month follow-up period. Lsg gave a false negative result in f per cent and a false positi e result in nine per cent. The examination failed technically either partly or completely in three cases chiefly because the patient mo ed. A repeat examination performed on two patients was completely satisfactory

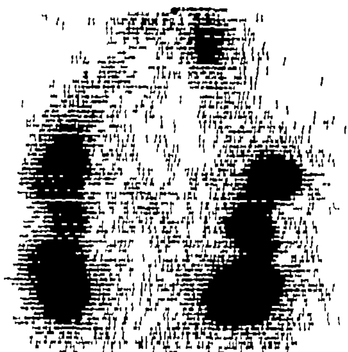


Fig. 4. Patient No. 7: a relative stasis above symphysis on the left side and a thickened channel crossing to the other side.

lymphography by no means may be considered a harmless measure (v. *Numers* 1965). At any rate it should not be performed on debilitated patients or patients with impaired pulmonary function (*Eiken* and *Madsen* 1965).

Hence lsg must be regarded as an easy and useful, supplementary diagnostic method for use alongside routine clinical examination, cavography and urography when seeking to establish the soft tissue spread of a malignant process in the pelvis or abdomen. It reveals above all the inguinal nodes, external iliac group, common iliac group and para-aortic group, which comprise the most important lymphatic area of dissemination of gynaecological cancer. Thus lsg is able to reveal clinically occult disease requiring additional radiotherapy or surgery, whether it is a primary tumour or a recurrence that is involved.

Sacral, hypogastric and parametrial nodes were demonstrated by *Howett* and *Greenberg* (1966) by direct lymphangiography

Rauramo, Lauri, Grönroos, Matti and Kyöstilä, Juhani. Acta obst. et gynec. scandinav. 47 517 1968
From the Department of Obstetrics and Gynaecology (Professor Lauri Rauramo) University of Turku Finland

PROGNOSIS OF CARCINOMA CORPORIS UTERI MANAGED BY PREOPERATIVE RADIUM TREATMENT

BY

LAURI RAURAMO, MATTI GRÖNROOS AND JUHANI KYÖSTILÄ

Introduction

It is now generally agreed that the application of a fixed treatment scheme is not practicable in the management of carcinoma corporis uteri. Treatment must be individual and depends on several factors such as the spread of the process, the patient s age, obesity and general condition, and on certain coincident diseases such as cardiovascular diseases and diabetes. Obviously however the attending physician should know what method of treatment or combinations of methods are most suitable and effective at a given time. This remains a controversial problem. The following methods may be considered for carcinoma corporis uteri hysterectomy and radical hysterectomy by the abdominal or vaginal route radiotherapy or combined radiotherapy and surgery

Material

The series consists of 269 women with adenocarcinoma in the corpus uteri treated in 1953–1965 at the Women s Clinic University Central Hospital Turku. The number of these cases available for five year evaluation was 162. Patients with combined carcinoma of the body and cervix obviously originating in endocervix and endometrial in situ lesions were not included in the material. The patients were classified clinically into two stages (I and II) according to international practice depending

The method was not useful for the visualisation of uterine lymphatics and lymph nodes On the other hand the collaterals between the main lymphatic trunks of the pelvis were often demonstrable their course being from the side of the stasis to the contralateral side. Radiotherapy seemed to weaken the flow in the lymphatics and the uptake of the isotope in the lymph glands.

REFERENCES

Eiken M and *Madsen V* Acta obst. et gynec. scandinav 44 45 1965
Gerr J and *Delouche G* J Radiol Electrol. 44 86 1963
Howett M and *Greenberg, A.* Obstet. Gynec. 27 392, 1966
Hreshchyshyn M and *Sheehan F* Am. J Obst. Gynec. 91 118 1965
Hultborn K. Larsson L. and *Ragnhult M* Acta radiol. (Stockholm) 43 139 1955
Kottmeier H L. Personal communication 1967
Lang, E. Radiology 74 71 1960
von Numers C Ann. Chir Gyn Fenn. 54 232, 1965
Vourilainen A. and *Wiljasalo M* Ann. Chir Gyn Fenn 54 268 1965
zum Winkel K. and *von Keiser D* Fortschr Röntgenstr 100 90 1964
zum Winkel K and *Scheer K* Minerva Nucl 9 390 1965

Received on Oct 6 1967

Rauramo, Lauri, Grönroos, Matti and Kyöstilä, Juhani. Acta obst. et gynec. scandinav. 47 517 1968
From the Department of Obstetrics and Gynaecology (Professor Lauri Rauramo) University of Turku Finland

PROGNOSIS OF CARCINOMA CORPORIS UTERI MANAGED BY PREOPERATIVE RADIUM TREATMENT

BY

LAURI RAURAMO MATTI GRÖNROOS AND JUHANI KYÖSTILÄ

Introduction

It is now generally agreed that the application of a fixed treatment scheme is not practicable in the management of carcinoma corporis uteri. Treatment must be individual and depends on several factors such as the spread of the process, the patient's age, obesity and general condition, and on certain coincident diseases such as cardiovascular diseases and diabetes. Obviously however the attending physician should know what method of treatment or combinations of methods are most suitable and effective at a given time. This remains a controversial problem. The following methods may be considered for carcinoma corporis uteri hysterectomy and radical hysterectomy by the abdominal or vaginal route, radiotherapy or combined radiotherapy and surgery

Material

The series consists of 269 women with adenocarcinoma in the corpus uteri treated in 1953–1965 at the Women's Clinic, University Central Hospital, Turku. The number of these cases available for five-year evaluation was 162. Patients with combined carcinoma of the body and cervix obviously originating in endocervix and endometrial *in situ* lesions were not included in the material. The patients were classified clinically into two stages (I and II) according to international practice depending

The method was not useful for the visualisation of uterine lymphatics and lymph nodes On the other hand, the collaterals between the main lymphatic trunks of the pelvis were often de monstrable their course being from the side of the stasis to the contralateral side Radiotherapy seemed to weaken the flow in the lymphatics and the uptake of the isotope in the lymph glands.

REFERENCES

Eiken M and *Madsen V* Acta obst. et gynec. scandinav *44* 45 1965
Gert J and *Delouche G J* Radiol Electrol *44* 86 1963
Hovett M and *Greenberg, A.* Obstet. Gynec. *27* 392, 1966
Hreshchyshyn M and *Sheehan F* Am. J Obst Gynec. *91* 118 1965
Hultborn K *Larsson L.* and *Ragnhult M* Acta radiol (Stockholm) *43,* 139 1955
Kottmeier H -L. Personal communication 1967
Lang, E. Radiology *74* 71 1960
von Numers C Ann Chir Gyn. Fenn. *54* 232, 1965
Vourilainen A. and *Wiljasalo M* Ann. Chir Gyn. Fenn *54* 268 1965
zum Winkel K. and *von Keiser D* Fortschr Röntgenstr *100* 90 1964
zum Winkel K. and *Scheer K.* Minerva Nucl *9* 390 1965

Received on Oct. 6 1967

Rauramo, Lauri, Grönroos, Matti and Kyöstilä, Juhani, Acta obst. et gynec. scandinav 47 517 1968
From the Department of Obstetrics and Gynaecology (Professor Lauri Rauramo) University of Turku Finland

PROGNOSIS OF CARCINOMA CORPORIS UTERI MANAGED BY PREOPERATIVE RADIUM TREATMENT

BY

LAURI RAURAMO, MATTI GRÖNROOS AND JUHANI KYÖSTILÄ

Introduction

It is now generally agreed that the application of a fixed treatment scheme is not practicable in the management of carcinoma corporis uteri. Treatment must be individual and depends on several factors such as the spread of the process, the patient s age, obesity and general condition, and on certain coincident diseases such as cardiovascular diseases and diabetes. Obviously however the attending physician should know what method of treatment or combinations of methods are most suitable and effective at a given time. This remains a controversial problem. The following methods may be considered for carcinoma corporis uteri. hysterectomy and radical hysterectomy by the abdominal or vaginal route, radiotherapy or combined radiotherapy and surgery

Material

The series consists of 269 women with adenocarcinoma in the corpus uteri treated in 1953–1965 at the Women s Clinic, University Central Hospital Turku. The number of these cases available for five-year evaluation was 162. Patients with combined carcinoma of the body and cervix obviously originating in endocervix and endometrial in situ lesions were not included in the material. The patients were classified clinically into two stages (I and II) according to international practice, depending

The method was not useful for the visualisation of uterine lymphatics and lymph nodes On the other hand, the collaterals between the main lymphatic trunks of the pelvis were often de monstrable their course being from the side of the stasis to the contralateral side. Radiotherapy seemed to weaken the flow in the lymphatics and the uptake of the isotope in the lymph glands.

REFERENCES

Eiken M and *Madsen V* Acta obst. et gynec. scandinav *44* 45 1965
Gerr J and *Delouche G J* Radiol Electrol *44* 86 1963
Howett M and *Greenberg, A.* Obstet. Gynec. *27* 392, 1966
Hreshchyshyn M and *Sheehan F* Am. J Obst. Gynec. *91* 118 1965
Hultborn K. Larsson L. and *Ragnhult M* Acta radiol. (Stockholm) *43* 139 1955
Kottmeier H-L. Personal communication 1967
Lang, E. Radiology *74* 71 1960
von Numers C Ann. Chir Gyn. Fenn. *54* 232, 1965
Vountilainen A. and *Wiljasalo M* Ann Chir Gyn. Fenn. *54* 268 1965
zum Winkel K. and *von Keiser D* Fortschr Röntgenstr *100* 90 1964
zum Winkel K. and *Scheer K.* Minerva Nucl. *9* 390 1965

Received on Oct 6 1967

Table II. Correlation Between the Clinical Stage and the Mode of Treatment

Clinical Stage	Mode of Treatment		
	Operation + Postoperative Intravag. Radium (+X-Rays)	Radium + X-Rays	Preliminary Radium + Operation (+X-Rays)
I/1	80	11	89
1/2		51	
II	8	26	4
Total	88	88	93

application. In addition, the patients were given X rays in the usual way after two weeks.

In the third group the patients were first given complete radium therapy by the Heyman-Kottmeier method described above and, 2 3 weeks after the second application, hysterectomy and removal of the adnexa were performed. If the surgical specimen showed that the carcinoma had spread halfway into the myometrium or further postoperative X rays were also administered.

Table II shows the distribution of the patients on the basis of the therapeutic method. The distribution was fairly even patients treated with operation and postoperati e radiotherapy totalled 88 (group A) patients given radiotherapy alone also totalled 88 (group B) patients given preliminary complete radium therapy followed by surgery totalled 93 (group C) Groups A and C include similar proportions of patients of St. I/1 whereas in group B St. I/1 cases are naturally few

Results

The crude five-year cure rate, *irrespective of the therapeutic method* in groups graded according to the degree of clinical spread was, as could be expected, best in cases of St. I/1 i.e 80 per cent (Table III) (the relative survival rate 87 per cent) Only 40 per cent of the patients of St. I/2 (Table IV) and 27 per cent of those of St. II (Table V) were alive after five years The

Table I Correlation Between the Age and Clinical Stage

Clinical Stage	Age Groups						
	30–39	40–49	50–59	60–69	≦70	Total	Mean Age
I/1	3	41	89	42	5	180	55.2
I/2	–	2	5	20	24	51	67.8
II	–	6	10	9	13	38	61.8
Total	3 (1%)	49 (18%)	104 (39%)	71 (26%)	42 (16%)	269	

on whether the growth was confined to the uterus or had spread outside it. Stage I was divided in two subgroups clinically oper able (1) and clinically inoperable (2) cases The latter subgroup consisted of the patients whom the anesthesiologist or internist classified as a definite operative risk The new recommendations for clinical classification adopted in 1961 in Vienna should be used from January 1 1962. Therefore, they were not used in the present material

The largest ten year age group consisted of patients aged 50–59 The *mean age was 58.5 years* the youngest patient was 36 and the oldest 87 The mean age of group St. I/1 was 55.2, of group St I/2 67 8 and of group St II 61 8 years (Table I)

Treatment

The patients were treated according to three therapeutic schemes

One group consisted of patients who had first had hysterec tomy and removal of the adnexa *followed by a single intravaginal* radium application and conventional X ray treatment.

The second group was given radium and X ray treatment alone According to *Heyman* s (1941) packing method the uterine cavity was filled twice with an interval of two weeks with small sources of irradiation so-called bolts The dose was calculated so that the dosage to the outer surface of the corpus uteri corresponded to about 3000 gamma roentgens (*Kottmeier* 1959) Intravaginal radium application was combined with the second

Table II. Correlation Between the Clinical Stage and the Mode of Treatment

Clinical Stage	Mode of Treatment		
	Operation + Postoperative Intravag. Radium (+X-Rays)	Radium + X-Rays	Preliminary Radium + Operation (+X-Rays)
I/1	80	11	89
I/2		51	
II	8	26	4
Total	88	88	93

application. In addition, the patients were given X-rays in the usual way after two weeks.

In the third group the patients were first given complete radium therapy by the Heyman-Kottmeier method described above and, 2 3 weeks after the second application, hysterectomy and removal of the adnexa were performed. If the surgical specimen showed that the carcinoma had spread halfway into the myometrium or further postoperative X-rays were also administered.

Table II shows the distribution of the patients on the basis of the therapeutic method. The distribution was fairly even patients treated with operation and postoperative radiotherapy totalled 88 (group A) patients given radiotherapy alone also totalled 88 (group B) patients given preliminary complete radium therapy followed by surgery totalled 93 (group C) Groups A and C include similar proportions of patients of St. I/1 whereas in group B St. I/1 cases are naturally few

Results

The crude fi e year cure rate irrespective of the therapeutic method in groups graded according to the degree of clinical spread was as could be expected, best in cases of St. I/1 i.e. 80 per cent (Table III) (the relative survival rate 87 per cent) Only 40 per cent of the patients of St. I/2 (Table IV) and 27 per cent of those of St. II (Table V) were alive after five years. The

Table I Correlation Between the Age and Clinical Stage

Clinical Stage	Age Groups						
	30–39	40–49	50–59	60–69	≥70	Total	Mean Age
I/1	3	41	89	42	5	180	55.2
I/2	–	2	5	20	24	51	67.8
II	–	6	10	9	13	38	61.8
Total	3 (1%)	49 (18%)	104 (39%)	71 (26%)	42 (16%)	269	

on whether the growth was confined to the uterus or had spread outside it. Stage I was divided in two subgroups clinically operable (1) and clinically inoperable (2) cases. The latter subgroup consisted of the patients whom the anesthesiologist or internist classified as a definite operative risk. The new recommendations for clinical classification adopted in 1961 in Vienna should be used from January 1 1962. Therefore, they were not used in the present material.

The largest ten year age group consisted of patients aged 50–59 The mean age was 58.5 years the youngest patient was 36 and the oldest 87 The mean age of group St. I/1 was 55 2, of group St. I/2 67.8 and of group St. II 61 8 years (Table I)

Treatment

The patients were treated according to three therapeutic schemes

One group consisted of patients who had first had hysterectomy and removal of the adnexa followed by a single intravaginal radium application and conventional X-ray treatment

The second group was given radium and X-ray treatment alone. According to *Heyman*'s (1941) packing method the uterine cavity was filled twice with an interval of two weeks with small sources of irradiation, so-called bolts The dose was calculated so that the dosage to the outer surface of the corpus uteri corresponded to about 3000 gamma roentgens (*Kottmeier* 1959) Intravaginal radium application was combined with the second

Table II. *Correlation Between the Clinical Stage and the Mode of Treatment*

Clinical Stage	Mode of Treatment		
	Operation + Postoperative Intravag. Radium (+X-Rays)	Radium + X-Rays	Preliminary Radium + Operation (+X-Rays)
I/1	80	11	89
I/2		51	
II	8	26	4
Total	88	88	93

application. In addition the patients were given X-rays in the usual way after two weeks.

In the third group the patients were first given complete radium therapy by the Heyman-Kottmeier method described above and, 2 3 weeks after the second application, hysterectomy and removal of the adnexa were performed. If the surgical specimen showed that the carcinoma had spread halfway into the myometrium or further postoperative X-rays were also administered.

Table II shows the distribution of the patients on the basis of the therapeutic method. The distribution was fairly even patients treated with operation and postoperative radiotherapy totalled 88 (group A) patients given radiotherapy alone also totalled 88 (group B) patients given preliminary complete radium therapy followed by surgery totalled 93 (group C). Groups A and C include similar proportions of patients of St. I/1 whereas in group B St. I/1 cases are naturally few

Results

The crude five-year cure rate *irrespective of the therapeutic method* in groups graded according to the degree of clinical spread was, as could be expected, best in cases of St. I/1 *i.e.* 80 per cent (Table III) (the relative survival rate 87 per cent) Only 40 per cent of the patients of St. I/2 (Table IV) and 27 per cent of those of St. II (Table V) were alive after five years. The

Table III *Results in Stage I/1 ("Operable")*

	Follow-Up Years				
	1	2	3	4	5
No of cases	180	167	153	137	111
Crude survival rate per cent	99	93	89	87	80
No of deaths from intercurrent disease	0	2	6	6	8

Table IV *Results in Stage I/2 ("Clinically Inoperable")*

	Follow-Up Years				
	1	2	3	4	5
No. of cases	51	44	37	28	25
Crude survival rate per cent	86	68	65	54	40
No of deaths from Intercurrent disease	5	10	10	9	11

Table V Results in Stage II (Inoperable)

	Follow-Up Years				
	1	2	3	4	5
No of cases	38	34	32	27	26
Crude survival rate per cent	68	47	34	30	27
No. of deaths from intercurrent disease	0	0	0	0	0

crude five year survival rate for the total series was 65 4 per cent (Table VI) (the relative survival rate 77 1 per cent)

Analysis of the five year cure results achieved by different therapeutic methods *independently of the degree of spread* of the disease (Tables VII VIII and IX) shows that all three methods of treatment have been used with practically equal frequency both group A and B comprised 88 patients and group C 93 patients Of these 64 55 and 43 patients respectively were available *for a five-year* follow up The proportion of five-year survivors in the group A was 75 per cent In the cases treated

Table VI *Results for All Patients Treated 1953–1965*

	Follow-Up Years				
	1	2	3	4	5
No. of cases	269	245	222	192	162
Crude survival rate per cent	92	83	77	76	65.4
No. of deaths from intercurrent disease	5	12	16	15	19

Table VII *Results for All Patients Treated with the Combination of Hysterectomy and Postoperative Irradiation (Group A)*

	Follow-Up Years				
	1	2	3	4	5
No. of cases	88	83	77	74	64
Crude survival rate, per cent	99	89	86	82	75
No of deaths from intercurrent disease	0	2	4	4	5

Table VIII *Results for All Patients Treated with Radium and X-Rays Only* (Group B)

	Follow-Up Years				
	1	2	3	4	5
No. of cases	88	79	73	58	55
Crude survival rate per cent	78	58	52	45	36
No. of deaths from intercurrent disease	5	10	11	10	13

Table IX *Results for All Patients Treated with the Combination of Radium and Postradiative Operation and in Some Cases X-Rays* (Group C)

	Follow-Up Years				
	1	2	3	4	5
No. of cases	93	83	72	60	43
Crude survival rate per cent	100	98	93	92	88
No. of deaths from intercurrent disease	0	0	1	1	1

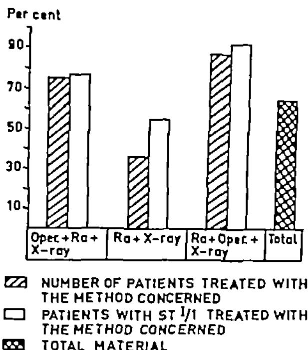


Fig 1 Five-year results obtained using various methods.

only with irradiation (group B) the percentage was 36 and in the group treated with primary irradiation and postradiative operation (group C) it was as high as 88 However these groups are not directly comparable as the severity of the disease varied from one group to another it was most severe in the group given radiotherapy alone

We therefore selected as the basis of our comparison of methods a population in which the disease displayed *the same degree of clinical spread* in all the patients The cases of *stage I/1* provided such a group for our purposes (Table X and Fig. 1) The crude five year cure rate for these patients in group A (operation + intravaginal radium + X-rays) was 77 ± 16 7 per cent The corresponding figure for group B (radium + X rays) was 55 ± 6.9 per cent and in group C (preliminary radium + operation + X rays) the five-year survival rate was as high as 93 ± 16 0 per cent. The last result was significantly better than the results

Table X. *Results in St U1 Using Various Methods of Treatment*

Mode of Treatment		Follow-Up Years				
		1	2	3	4	5
Group A	No of cases	80	76	73	69	60
	Crude survival rate, per cent	99	90	86	84	77
Group B	No of cases	11	11	11	11	11
	Crude survival rate per cent	100	73	64	64	55
Group C	No. of cases	89	80	69	57	40
	Crude survival rate per cent	100	99	96	95	93

Table XI. *Five-Year Survival Rate of All Patients Treated in Various Periods*

	Therapy Given in the Years		
	1953–55	1956–58	1959–61
No of cases	57	42	62
Crude survival rate per cent	53	67	81
No of deaths from intercurrent disease	7	6	4

achieved with the other two methods $(0.01 > p > 0.00125)$ The results achieved in groups A and B did not differ significantly from each other $(0.10 > p > 0.05)$

Another pointer to the superiority of preoperative radium therapy is the five-year survival rate calculated according to the period at which the treatment was given (Table XI) In the early years of our therapeutic series the material consisted chiefly of patients treated primarily by operation whereas towards the end patients gi en preliminary radium therapy became more common. The first patients given pre-operative radium were from 1957 in 1956–1958 they accounted for 20 per cent and in 1959–1961 for 46 per cent of all the patients treated in the respecti e years. The crude five-year cure rate of the patients treated in 1953–1955 was only 53 ± 6.7 per cent. The correspond

ing figure for the next three-year period was 67 ± 7 4 per cent and in the last three-year period it was 81 ± 5 0 per cent. The cure results in the middle three year period did not differ from either of the other three year periods (0 10 > p > 0 05) but the results for the last period were significantly better than those for the first period (0 0025 > p > 0 0005)

Discussion

Results of irradiation or operative therapy of carcinoma corporis uteri in Scandinavian countries have been published by *e g Turtola* (1947) *Wetterdal* (1958) *Kottmeier* (1959) *Ojanen et al* (1961) and *Koller* (1963 and 1967) *Rauramo* (1964 and 1967) has presented preliminary results concerning the preoperative radium treatment.

Half of *Turtola s* material 74 patients were treated radiologically and the other half operatively mostly by hysterectomy and removal of the adnexa In radiotherapy die alte Methode was used in which ein Radiumrohrchen is applied in the uterine cavity and a plaque against the portio Most patients received in addition X rays as after treatment. The five-year survival rate *for the irradiated patients was 31 and for the operative cases* 71 4 per cent

It appears to be the consensus in the literature that the results achieved by the intracavitary packing method of *Heyman* (1941) and *Kottmeier* (1959) are better than those of the old tandem technique The five-year cure rate with *Kottmeier s* packing method in 1948–1951 was 66 4 per cent and in the total series in 1936–1951 it was 63.3 per cent The results were thus of the same order as our own, 65 4 per cent.

In contrast, *Wetterdal gives operative therapy definite prefer* ence over radiotherapy The 69 patients with primary operation who were available for five year follow up represented a cure rate of 86 per cent and the 58 irradiated patients correspondingly a cure rate of 64 per cent.

The series reported by *Ojanen et al* consisted of 262 patients who were operated on and 152 patients treated with radium and X rays The authors regarded abdominal *total hysterectomy with*

removal of the adnexa as the most appropriate operation for carcinoma corporis uteri. In the former population the five year survival rate was 77.2 per cent and in the latter only 45.8 per cent. However the authors drew no conclusions as to the superiority of the methods since there is an element of selection, the most favourable being operated upon.

A more reliable basis for comparison in fact, is to distribute the patients according to international classification into stages on the basis of the spread of the tumour. *Kottmeier*—using the packing method—reported the cure rate to be 84.6 per cent for clinically operable cases 49.7 per cent for clinically inoperable cases and 27.4 per cent for inoperable cases. Our own results were roughly similar 80 40 and 27 per cent, respectively. The effectiveness of the different methods can be studied best, in our opinion, if they are used on patients of the population representing the same degree of spread. In our own material, cases of St. I/1 were treated in three ways patients of group A were managed by combining hysterectomy with removal of the adnexa and postoperative irradiation group B received radium and X-ray therapy alone In group C the packing method was used followed by total hysterectomy with removal of the adnexa and at times X-rays. *Administration of preoperative radium improved the results significantly the five-year survival rate was 93 per cent.* In *Koller's* (1963) material the patients subjected to preoperative radiation showed a tendency to lower survival rate than the cases with postoperative irradiation. This may according to the author be accounted for by the increasing trend in recent years to reserve this type of treatment for older patients.

We consider in agreement with *Sjövall* (1958) *Montgomery et al.* (1960) and several other authors that preoperative radium improves considerably the results achievable in treating corpus carcinoma. It devitalises the carcinoma primarily thus preventing manipulative spread and local recurrences. In *Wetterdal's* and several other authors opinion, however irradiated tissue is perceptibly more difficult to operate on than normal tissue. But several authors contend that the changes caused by irradiation, first and foremost congestion and oedema disappear within six weeks of radiotherapy and this period has therefore been re-

garded as optimal for operative therapy *Decker* (1958) regarded six weeks as too long because the carcinoma tissue that might occur outside the uterus, does not receive a cancerocidal dose from radium application and the process can grow rapidly in the course of this period. The author proposed, in fact, an interval of one week between radium therapy and surgery No technical surgical difficulties were encountered at that time. The slightly increased oedema was deemed to be actually beneficial and there was hardly any increase in bleeding tendency The interval between the last application of radium and surgery at our clinic has been 2–3 weeks We consider this fairly suitable as oedema and congestion have already disappeared and no scar tissue has formed by then.

SUMMARY

The results for 269 patients with carcinoma corporis uteri treated at the Women's Clinic University Central Hospital Turku in 1953–1965 are analysed Patients of St. I/1 distributed into three groups constituted the basis of the comparison. The patients of the first group had a preliminary operation followed by intravaginal radium and in some cases also X rays the patients of the second group received radium and X-rays alone and the patients of the third group were treated by the radium packing method followed by operation and, in some cases X rays The improved results from the administration of preoperative radium were statistically highly significant and the crude five year cure rate was 93 per cent For the patients treated by preliminary operation the corresponding rate was 77 per cent, and those given radiotherapy alone 55 per cent. The crude five-year cure rate for all the patients was 65 4 per cent (the relative survival rate 77 1 per cent) The crude five year survival rate for all patients during the period when preoperative radium therapy was given systematically was 81 0 per ent

REFERENCES

Decker W H. Am. J Obst. & Gynec. 76 20 1958

Heyman, J Reuterwall O and Benner S Acta radiol. 22 11 1941

Koller O presented at the International Symposium on End Results of Cancer Therapy Sandefjord, Norway September 1963

presented at the Fifth World Congress of Gynaecology and Obstetrics Sydney September 1967

Kottmeier H-L. Am. J Obst. & Gynec. 78 1127 1959

Montgomery J B Long, W R. Farell D M. and Helm G A., Am. J Obst. & Gynec. 80 972, 1960

Oysnen R. Turtola V and Olki M. Ann. Chir Gyn. Fenn. Suppl. 100 1961

Rauramo L. Föredrag vid Suomen Gynekologiyhdistyksen jatkokoulutuspäivät, Helsinki, 1964

presented at the Fifth World Congress of Gynaecology and Obstetrics Sydney September 1967

Sjövall A Förhandlingar vid Nord. För Obst. och Gynek. Stockholm 28-30 aug 1958

Turtola V Ann. Chir Gyn Fenn., 36 47 1947

Westerdal P Förhandlingar vid Nord. För Obst. och Gynek., Stockholm, 28-30 aug 1958

Received on Oct. 6 1967

Rauramo, L., Grönroos, M and Kivikoski, A Acta obst. et gynec. scandinav 47 528, 1968
From the Department of Obstetrics and Gynaecology (Professor Lauri Rauramo) University of Turku Turku Finland

EXPERIENCE OF THE USE OF 6α-METHYLLYNESTRENOL FOR PATIENTS WITH GENITAL CARCINOMA

BY

L. RAURAMO M. GRÖNROOS AND A. KIVIKOSKI

The possible therapeutic effect of steroids principally oestrogens or gestagens in the treatment of carcinoma of the genitalia has begun to attract attention again in the last few years. It has been suggested in the literature that gestagens might cause some cases of disseminated carcinoma of the corpus to regress (*Kaiser* 1959 *Scherman* and *Woolf* 1959 *Kelly* and *Baker* 1961 *Stoll* 1961 *Kottmeier* 1962 *Went* 1964 *Curchod* 1965 *Jensen* and *Lass* 1965) On the other hand the results have not been equally favourable in carcinoma of the cervix For example, *Hertz* and *Cromer* (1954) retracted their earlier report of the beneficial influence of progesterone on the healing of carcinoma of the cervix. *Barnes* and *Rothchild* (1953) were likewise unable to establish retardation of the growth of carcinoma of the cervix in patients with recurrent tumour Intravenous progesterone therapy was not found by *Rothchild et al* (1955) to affect the growth of carcinoma of the cervix.

Rauramo et al (1964) reported that of postmenopausal women with carcinoma of the cervix those that still displayed some oestrogen activity had a less favourable prognosis. Furthermore, *Rauramo* and *Grönroos* (1963) established that postmenopausal patients with carcinoma of the cervix excreted smaller amounts of oestrogens in a tolerance test than a control group. Studying the radiation response (RR) *Rauramo* and *Kangas* (1964) also found that the patients with a weak RR and also a poorer prognosis were generally those showing relatively strong oestrogen activity

Table I *Mean Values of Haemoglobin Erythrocytes Leucocytes and Thrombocytes Weekly from the Beginning of the Therapy*

Blood Values	Weeks I	II	III	IV	V	VI	VII	VIII	IX	X
Hb (Gm/100 ml)	12 0	11 2	11 2	11 2	11 7	11 7	11 8	11.8	11.5	11.6
RBc (10^6/cu mm)	4 1	3 7	3 7	3 7	4 0	3 9	3 9	3 8	3 9	3 8
WBc/cu mm	8270	7850	6850	6270	6200	6750	6400	5850	6110	6250
Platelets/cu mm	304370	306320	313080	310360	308540	200770	288800	283440	324590	269560

Table II. *Liver Function Tests*

A = before therapy and 1 month after methyllynoestrenol (10 mg/24 hr) + radiation therapy
B = after 1 month radiation therapy

A. Patient No	37	38	39	40	41	42
SGOT (I U)	25	25	55	40		20
Before therapy Bilirubin (mg %)	0.4	0 9	0 4	0 3	0 4	0.5
SGOT (units)	30	20	60	35	30	20
After therapy Bilirubin	0.3	0.5	0.5		0 4	0.2

B. Patient N	43	44	45	46	47	48	49	50	51
SGOT		30	20	15	20	15	15	30	20
After radiation Bilirubin	0.6	0.3	0.4	0 6	0.3	0 4	0.4	0 4	0.6

Table III. *Urinary Excretion of Oestrogens (µg/24 hs) Before and After 1 Month's Methyllynestrenol and Radiation Therapy*
A. *Basal Excretion*

Patient No	Age	Before Therapy				After Therapy			
		Oestrone	Oestradiol	Oestriol	Total Oestrogens	Oestrone	Oestradiol	Oestriol	Total Oestrogens
A. Basal Excretion									
1	50	4.8	1.0	23.5	29.3	6.1	4.0	12.5	22.6
3	41	10.1	3.9	25.5	39.5	25.1	6.1	43.6	74.8
5	50	4.7	2.5	17.0	24.2	5.5	3.4	10.0	18.9
6	54	4.2	2.2	21.5	27.9	1.1	0.5	1.3	2.9
7	51	1.4	1.0	1.1	3.5	5.1	2.8	5.7	13.6
8	51	1.2	0	25.8	27.0	2.8	2.5	3.5	8.8
9	51	1.1	0	3.5	4.6	0.3	0	7.9	8.2
10	41	2.6	4.3	7.7	14.6	6.5	5.0	12.5	24.0
11	43	18.3	0.7	31.2	50.2	1.6	0.8	2.5	4.9
12	40	1.6	1.0	5.7	8.3	0	2.9	2.7	5.6
13	37	5.1	0.1	8.5	13.7	12.8	7.9	5.0	25.7
15	35	1.3	2.6	16.6	20.5	9.5	1.9	4.8	16.2
16	74	0	0	2.1	2.1	8.2	0	6.2	14.4
18	53	0	0	3.0	3.0	1.4	0.6	11.9	13.9
19	63	0	0	3.8	3.8	1.2	2.4	13.4	17.0
22	66	0.5	0	1.9	2.4	0.6	0.5	4.8	5.9
24	55	2.9	1.8	5.2	9.9	6.7	0.9	13.9	21.5
25	66	1.3	0.5	13.6	15.4	2.9	1.5	4.3	8.7
26	61	2.8	0	9.3	12.1	11.7	0.9	20.0	38.6
34	60	5.0	1.4	7.6	14.0	8.4	1.0	6.0	15.4
Mean		3.4	1.2	11.7	16.3	5.9	2.3	9.6	18.2

B *Excretion After Tolerance Test (Dimenformon)*

3	41	48.9	27.6	53.8	132.3	49.3	29.1	28.8	107.2
9	51	46.6	37.2	19.9	103.7	72.3	45.2	30.0	147.5
11	43	75.5	12.9	55.4	143.8	19.1	8.1	17.0	44.2
15	35	10.7	1.4	139.3	151.4	44.5	23.0	69.5	137.0
16	74	12.4	1.7	40.3	54.4	64.5	32.5	12.5	109.5
18	53	26.1	11.8	50.3	88.2	27.5	18.2	13.4	59.1
19	63	5.8	3.6	49.3	58.7	75.9	41.7	70.8	188.2
22	68	4.0	1.4	51.0	56.4	116.5	2.6	101.2	220.3
26	61	17.2	2.5	46.8	68.5	30.0	1.1	34.6	65.7
Mean		27.5	11.1	56.5	95.0	55.5	22.4	42.0	119.9

In their summary of the relationship between oestrogens and carcinoma *Diczfalusy* and *Lauritzen* (1961) mentioned that oestrogens have not been shown to have any actual cancerogenic effect in man but that in both normal and cancer cells they stimulate growth They also mentioned that pregnancy has as is well known an aggravating effect on almost all carcinomas with rapid deterioration occurring in the puerperium.

According to these authors there is a continuous marked increase in oestrogen secretion until the end of pregnancy after which it diminishes and the normal level of a non-pregnant woman is reached by the fifth day of the puerperium. As the spread of a carcinoma during pregnancy takes place especially in the course of the puerperium when the high oestrogen secretion of pregnancy has ceased, it can be argued that the poorer prognosis of those cervical carcinoma patients who had displayed oestrogen activity before treatment might also be due to the cessation of ovarian function and the decrease in oestrogen secretion resulting from the carcinoma therapy The main factor here would be an increase in gonadotrophin secretion because of a decrease in oestrogen levels in the blood The reports of poor results achieved in the treatment of carcinoma of the cervix with progesterone show that no adequate tests have been performed so far to clarify this issue If the aim were to eliminate some of the disadvantages of the absence of oestrogen, long term and intensive steroid therapy should be administered, and it should be instituted before the beginning of other carcinoma therapy Most of the steroid therapeutic trials to date have been conducted with incurable cases first treated a long time previously

In our search for a suitable steroid for our therapeutic trials we decided upon 6α methyllynestrenol (17α-ethinyl-6α-methyl-estr 4-ene-17β-ol) This steroid which is still in the experimental stage, has been found to have the following effects when administered orally 1 pronounced antiovulatory effect 2. antioestrogenic effect 3 A progestational effect can be provoked by large doses but none was demonstrated with the doses which are sufficient to inhibit ovulation and cause the antioestrogenic effect.

Research preparation of N V Organon, Netherlands

Table IV *Urinary Excretion of Gonadotrophins (M.U.) Before and After 1, 2 and 3 Months' Methyllynestrenol (10 mg/24 hr) and Radiation Therapy*

Patient No.	Age	Before Therapy	After 1 Month	After 2 Months	After 3 Months
1	50	22–45	45		
5	50	22–45	22–45		
6	54	22	45–90		
7	51	45–90	45		
8	51	22	22		
12	49	45	45		
15	35	22–45	22		
17	46	22–45	45–90		
24	55	22	22–45		
37	41			45–90	45
38	44			45–90	22
39	33			22	22
40	44			22–45	45–90
41	45			45	22–45
42	41			22	7–22
52 No hormone therapy	45			7–22	7
53 No hormone therapy	43			22	22
54 No hormone therapy	48			22	22

Material and Methods

The material consisted of a total of 64 women with genital carcinoma who had been treated by radiation or cytotoxic drugs or both. Forty-two of these patients were given methyllynestrenol tablets by mouth in doses of 10 mg daily for 3 months. 10 patients, 4 of whom had lung metastases, received 40 mg daily for 3 months. During this time the patients' subjective condition was followed and the haemoglobin, erythrocyte, leucocyte and thrombocyte values were determined weekly. The patients' age range was 32–76 years.

Urinary gonadotrophins (M.U.) were determined in 9 patients immediately before treatment and after 1 month of methyllynestrenol (10 mg/24 hours). In addition, the gonadotrophin values for 6 other patients were determined 2 and 3 months after the beginning of steroid therapy and for 3 control patients who did

Table V *Results After 3 Months Methyllynestrenol (10 mg/24 hr) and Radiation Therapy*

Patient No	Age	Diagnosis	Time of Observation		Symptom-less	Death
			Years	Months		
1	50	Ca cervicis ut. gr I	2	0	x	
2	51	"	2	1	x	
3	41		2	4	x	
4	51		2	1	x	
5	50		2	0	x	
6	54		1	6	x	
7	51		2	3	x	
8	51		2	2	x	
9	51	Ca cervicis uteri gr II	2	3		x
10	41		1	3	persist	
11	43	"	2	5	x	
12	49	"	2	0	x	
13	37		2	2	x	
14	60	Ca cervicis uteri gr III		7		x
15	35	"	1	8		x
16	74	"	1	6		x
17	46		1	7	x	
18	53			11		x
19	63		2	4	x	
20	49	Ca cervicis uteri gr IV	3	0	x	
21	32		1	2		x
22	66			5		x
23	72	Ca corporis uteri	2	8	x	
24	55		2	1	x	
25	66		2	5	x	
26	61		2	5	x	
27	50	Pseudomyxoma peritonei	3	3	x	
28	76	Tumor hormonalis ov. inoper		5		x
29	58	Ca cervicis ut. gr I resid	1	2		x
30	44	Ca cervicis ut. gr II resid.		4		x
31	71	Ca cervicis ut. gr II resid.	2	5		x
32	50	Ca cervicis ut. gr III resid	3	0		x
33	56	Ca port. gr II resid.		3		x
34	60	Ca corp. ut. resid		6		x
35	67	Ca corp. ut resid (+Ca mammae)		2		x
36	61	Ca ovarii l. a. c. metast		1		x
37	41	Ca cervicis ut. gr I	1	0	x	
38	41		1	0	x	
39	44	Ca cervicis ut. gr III		10	x	
40	33	Ca cervicis ut. gr I	1	1	x	
41	44	Ca cervicis ut. gr I	1	0	x	
42	45	Ca corporis uteri				
		Mean	1	10		

Table VI *Urinary Gonadotrophins SGOT and Serum Bilirubin During the Long-Term and High-Dosage Methyllynestrenol Therapy (40 mg/24 hr) During and After Radiation Treatment Age of Patients 40–45 Years*

Patient No	Gonadotrophins (M U)				SGOT (M.U)				Serum Bilirubin (mg %)			
	before the teroid therapy	months later			before therapy	months later			before therapy	months later		
		1	2	3		1	2	3		1	2	3
58	90	22–45	45	45	8	15	12	10	0.5	0 4	0.5	0.4
59	7 22	45–90	90	22–45	13	14	14	16	0.5	0 6	0 9	0.3
60	22–45	22–45	45	22–45	10	23	17	22	0 5	0 3	1.8	0.6
61	7–22	22–45	22	45	8	8	3	4	0 1	0 4	0 4	0.5
62	22				14				0 4			
63	90–180	90–180			6	9	12		0 7	1 1	0 4	

not receive any tablets There was furthermore a group of 6 patients given long term and high-dosage steroid therapy (40 mg/24 hours) whose gonadotrophins were determined prior to therapy and after 1 2 and 3 months of treatment. Gonadotrophin secretion was thus followed in a total of 24 patients.

Immediately before therapy and a month after it, the three classical oestrogens oestrone oestradiol and oestriol were determined from a 48-hour urine specimen by the method of *Brown* (1955) modified by *Diczfalusy* and *Westman* (1956) and *Brown et al* (1957) This basal secretion was determined for 20 patients Tolerance tests were performed in addition on 9 of the latter patients by administering a Dimenformon (oestradiol benzoate) ampoule intramuscularly before methyllynestrenol therapy and collecting the 48-hour urine subsequently The same procedure was repeated after 1 month of steroid therapy

Liver function was followed by determining the serum bilirubin (mg %) and SGOT (Table II gives the values in old international units and Table VI in micro units)

Results

a) *Pilot test* (Table I)

The mean haemoglobin values varied from 11.2 to 12.0 g% during 3 months of methyllynestrenol therapy No definite falling or rising trend was noted The erythrocyte count showed average variation over the range 3 7–4 1 million per cu.mm. The mean haemoglobin concentration was 28–32 % The leucocyte values displayed a falling trend after averaging 8,300 and 7 850 per cu.mm in the first weeks they had dropped to 5 850–6,250 by the eighth and tenth week. The thrombocyte values also appeared to fall to some extent. There was a gain in the patients mean weight in the course of 3 months 62–70 kg No subjective ill-effect from the tablets was reported.

b) *Effect on liver function* (Tables II and VI)

To study the liver function the serum bilirubin and SGOT were determined at the beginning of therapy and after 1 month s

course of treatment for 6 patients given methyllynestrenol (10 mg/24 hours)+radiation therapy and for 9 patients receiving only radiation therapy (Table II) The same values were deter mined for 6 patients after 1 2 and 3 months of methyllynestrenol (40 mg/24 hours) treatment (Table VI) The later samples displayed no rising trend.

c) *Effect on the urinary excretion of oestrogens with and without oestrogen tolerance test* (Table III)

Possible changes during methyllynestrenol therapy were studied by calculating the basal excretion before and after treatment and after the tolerance tests.

According to the basal excretion values the excretion of oestrone was an average of 2.5 μg/24 hours higher than before methyllynestrenol therapy the difference was statistically signi ficant (p=0 05) Oestradiol excretion was correspondingly 1 1 μg/24 hours higher (p=0 01) Oestriol seemed to decrease but no statistically significant difference was observed. Nor was there any significant change in total oestrogens The excretion values for each patient and the mean excretion levels are shown in Table III.

In the tolerance tests, the excretion of oestradiol increased by an average of 11.3 μg/24 hours (p=0 025–0.05) after methyllynestrenol therapy On the other hand no statistically significant changes occurred in the excretion of oestrone oestriol and total oestrogens.

d) *Effect on the urinary excretion of gonadotrophins* (Tables IV and VI)

As regards th 9 patients whose urinary gonadotrophin level was determined again after a 1-month course of therapy (10 mg/24 hours) excretion had increased in 4 fallen in 2 and remained unchanged in 3 The excretion decreased in 4 increased in 1 and remained unchanged in 1 of the patients whose gonadotrophin excretion values were compared after 2 and 3 months of treat ment Excretion was practically unchanged in 2 and decreased in 1 of the 3 patients not gi en methyllynestrenol therapy There

was no consistent falling tendency in the gonadotrophin levels even in the group given the higher dose (Table VI)

e) *Therapeutic effect* (Table V)

With the exception of 3 cases the observation period was under 3 years The average follow up period was 1 year 10 months and the longest 3 years 3 months All 12 patients with cervical carcinoma of stage I remained symptomless throughout the follow-up period. Three of the 5 patients in stage II were free from symptoms the carcinoma persisted in 1 case and 1 patient died. Three of the 7 cases in stage III were symptomless and 4 died during the observation period There were only 3 patients with carcinoma of the cervix of stage IV 1 of them survives and is free from symptoms (follow up period 3 years)

The patients with carcinoma of the corpus 5 in all remained symptom free throughout the follow-up period. Of the remaining 11 patients who also received 10 mg/24 hours the majority were cases of recurrent cervical carcinoma 1 was a case of pseudomyxoma peritonei and 1 a hormone-producing ovarian tumour Only 1 of these patients was symptomless at the end of the follow up period and the others died.

Four of the patients with carcinoma of the cervix had lung metastases and received 3 months methyllynestrenol therapy (40 mg/24 hours) The treatment was not found to inhibit the growth and spread of the metastases

Conclusions and Discussion

The patients tolerated 6α methyllynestrenol well. The haemoglobin, erythrocyte and mean haemoglobin values remained roughly within the normal range throughout the treatment. The leucocyte and thrombocyte values displayed a falling trend but this was most probably due to the concurrent radiotherapy administered to the majority of the patients. The slight weight gain may be attributable to the fluid-retaining effect of steroid. No changes were demonstrated in the liver function.

Examination of the effect of methyllynestrenol on the basal urinary excretion of oestrogens showed that the excretion of

oestrone was higher on average than before therapy Oestradiol excretion also increased. No statistical differences were established in excretion of the other oestrogens A comparison of excretion in the tolerance test before and after the course of methyllynestrenol showed that the oestradiol level had risen, on average 11.3 μg/24 hours It is obvious that the steroid under trial did not cause retention of oestrogens On the contrary the excretion of biologically active fractions increased. It must be remembered that the steroid itself may have been converted into oestrogens. The present investigation leaves this possibility open.

Short-term administration of the steroid seemed to have no clear influence on gonadotrophin excretion. After longer courses of treatment, on the other hand, there appeared to be a falling tendency though not a consistent one.

The observation period was an average of 1 year 10 months for the 42 patients given 10 mg/24 hours which precludes reliable conclusions as to the therapeutic effect. It would seem that steroid had no curative effect on the severest forms of carcinoma of the cervix. It may not even prolong life. Nor was it possible to demonstrate a curative effect on recurrent cervical carcinoma. In contrast, all the cases of carcinoma of the corpus remained symptomless throughout the observation period. The effect of methyllynestrenol therapy (40 mg/24 hours) was followed also in 4 patients with lung metastases who had a history of cervical carcinoma The 3-month course of treatment did not reduce the spread or incidence of the metastases.

SUMMARY

The effect of 6α-methyllynestrenol on the course of the disease in 52 patients with genital carcinoma and its effect on oestrogen and gonadotrophin excretion and liver function was studied as a pilot test. The patients tolerated the steroid well. Excretion of oestrone and oestradiol increased during the course of treatment. No definite changes were demonstrated in liver function, haematological indices or gonadotrophin excretion. No curative effect was established in the patients with carcinoma of the cervix or pulmonary metastases, but all the patients with carcinoma of the corpus remained symptomless throughout the follow-up period.

was no consistent falling tendency in the gonadotrophin levels even in the group given the higher dose (Table VI)

e) *Therapeutic effect* (Table V)

With the exception of 3 cases the observation period was under 3 years The average follow up period was 1 year 10 months and the longest 3 years 3 months. All 12 patients with cervical carcinoma of stage I remained symptomless throughout the follow up period. Three of the 5 patients in stage II were free from symptoms the carcinoma persisted in 1 case and 1 patient died. Three of the 7 cases in stage III were symptomless and 4 died during the observation period. There were only 3 patients with carcinoma of the cervix of stage IV 1 of them survives and is free from symptoms (follow up period 3 years)

The patients with carcinoma of the corpus 5 in all remained symptom free throughout the follow up period. Of the remaining 11 patients who also received 10 mg/24 hours the majority were cases of recurrent cervical carcinoma 1 was a case of pseudomyxoma peritonei and 1 a hormone producing ovarian tumour Only 1 of these patients was symptomless at the end of the follow up period and the others died.

Four of the patients with carcinoma of the cervix had lung metastases and received 3 months methyllynestrenol therapy (40 mg/24 hours) The treatment was not found to inhibit the growth and spread of the metastases

Conclusions and Discussion

The patients tolerated 6α-methyllynestrenol well. The haemoglobin erythrocyte and mean haemoglobin values remained roughly within the normal range throughout the treatment The leucocyte and thrombocyte values displayed a falling trend, but this was most probably due to the concurrent radiotherapy administered to the majority of the patients The slight weight gain may be attributable to the fluid retaining effect of steroid. No changes were demonstrated in the liver function.

Examination of the effect of methyllynestrenol on the basal urinary excretion of oestrogens showed that the excretion of

Acknowledgements

We are indebted to the Sigrid Juselius Foundation for a grant towards the costs of this investigation

REFERENCES

Barnes A. C. and *Rotchild J* Obst. et Gynec. *1* 147 1953
Brown J B Biochem. J *60* 185 1955
Brown J B Bulbrook R. D and *Greenwood F C* J Endocr *16* 49 1957
Curchod A. Schweiz. Med. Wschr 95 498 1965
Diczfalusy E. and *Lauritzen C.* Oestrogene beim Menschen. Springer Berlin Göttingen Heidelberg, 1961
Diczfalusy E. and *Westman A.* Acta Endocr (Kbh) *21* 321 1956
Hertz R and *Cromer J K.* J.A.M.A. *154* 1114 1954
Jensen S T and *Lass F* Ugeskr Laeg *41* 1294 1965
Kaiser R. Arch. f Gynäk. *193* 195 1959
Kelly R. M and *Baker W H* New Engl J Med *264* 216 1961
Kottmeier H L. Geburtsh. u. Frauenh 22 1070 1962
Rauramo L. and *Grönroos M* Ann. Chir Gyn. Fenn. 52 351 1963
Rauramo L Grönroos M and *Kivikoski A.* Ann. Chir Gyn. Fenn. *53* 110 1964
Rauramo L. and *Kangas S* Ann. Chir Gyn. Fenn. 53 120 1964
Rothchild J Hendricks C H Rosenblum J von Haam E. and *Barnes A. C* J Clin Endocr *15* 151 1955
Sherman A. J and *Woolf R. B* Am J Obst. Gynec. 77 233 1959
Stoll B A. Can chem. Rep *14* 83 1961
Wentz B W Obst. and Gynec *24* 370 1964

Received on Oct 6 1967